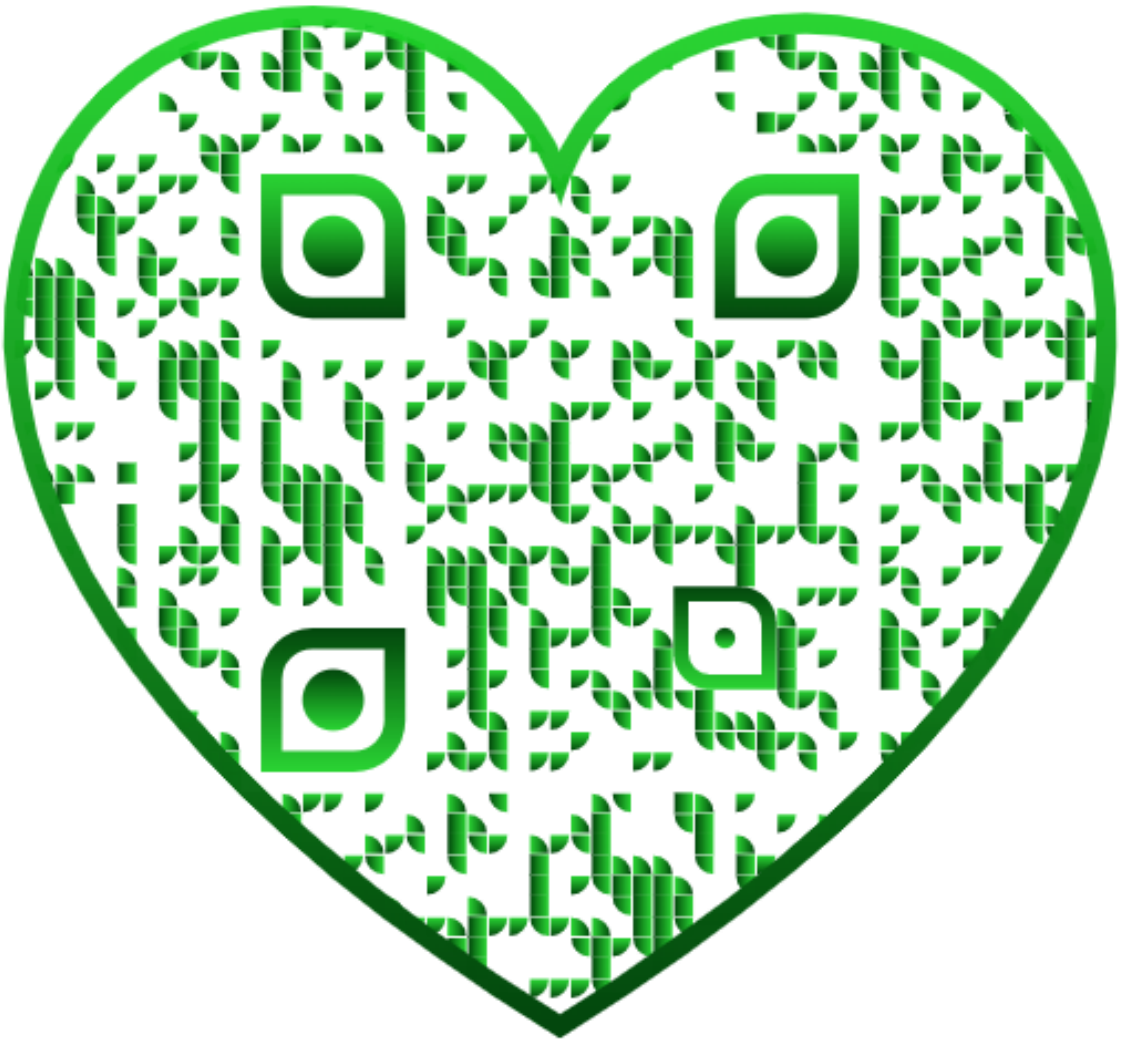
PRACTICAL MICROBIOLOGY FOR MEDICAL THIRD STUDENTS

Part 1

By Dr/Abeer Nasher

Lecturer in the Department of Medical Microbiology and Immunology



Dr/Abeer Nasher

Introduction to Microbiology Lab(1)

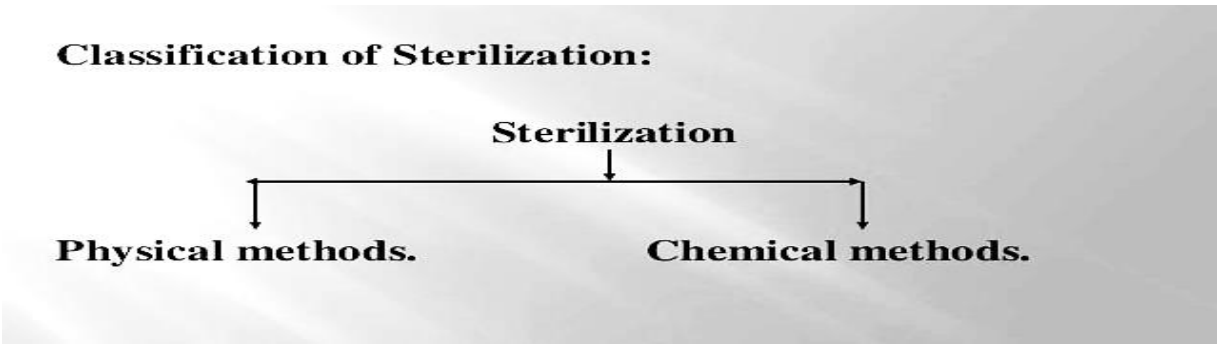
What is a microbiology lab.?

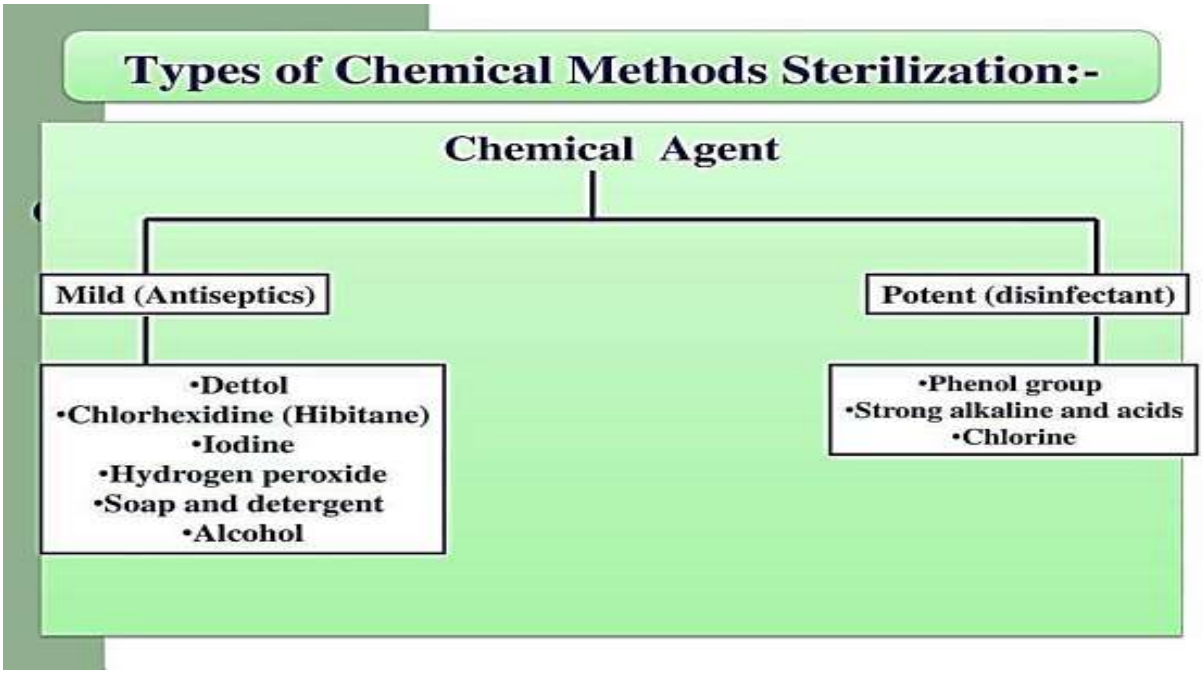
A microbiology laboratory is a laboratory devoted to the *culturing*, *examination*, and *identification* of microorganisms including bacteria, fungi, yeasts**.**

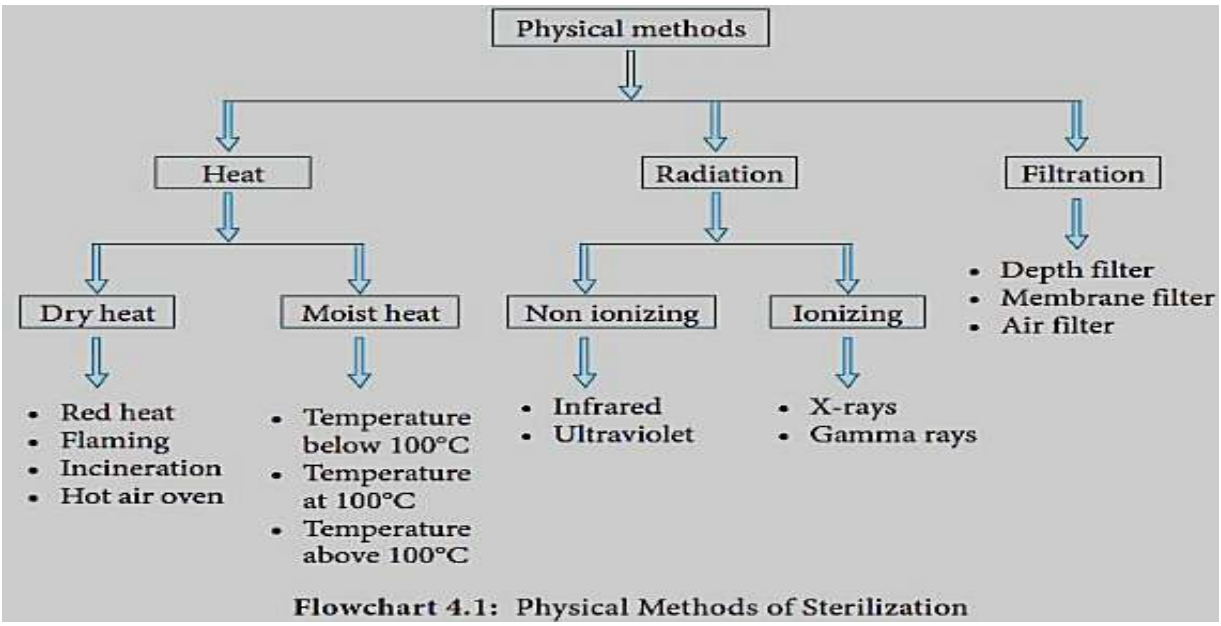
Safety and Security in a Microbiology Laboratory

1. It is forbidden to bring drinks and food into the laboratory and to avoid touching the mouth and nose at work
2. Wearing unopened shoes when entering the laboratory.
3. Always wear the **lab coat** inside the laboratory.
4. Wearing Gloves and handling with great care the pathogenic microbes.
5. Wearing masks.
6. Clean the bench before work and disinfect it with a disinfectant
7. Wash hands with soap and water before starting and after work.
8. Cultivation of samples inside the Safety Cabinet.
9. Disposal of medical specimens implanted ,needles, pollination of loops and swabs intended for cultivation in the waste intended for them.
10. Make sure to write the information of the cultured sample on Petri dishes (on cap) and on tubes for liquid environments

Types Of Sterilization Methods

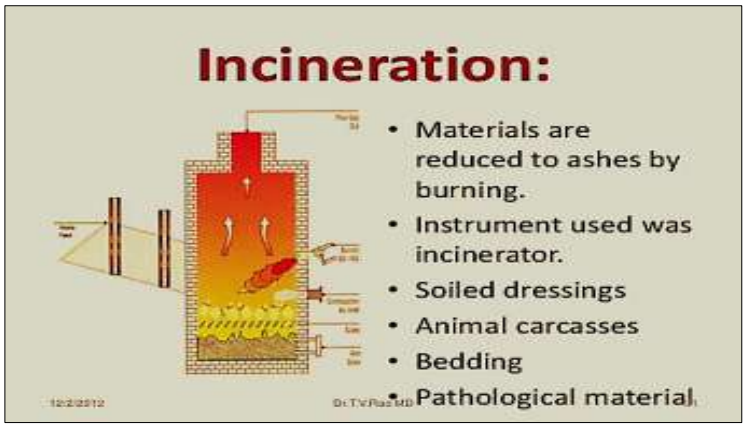


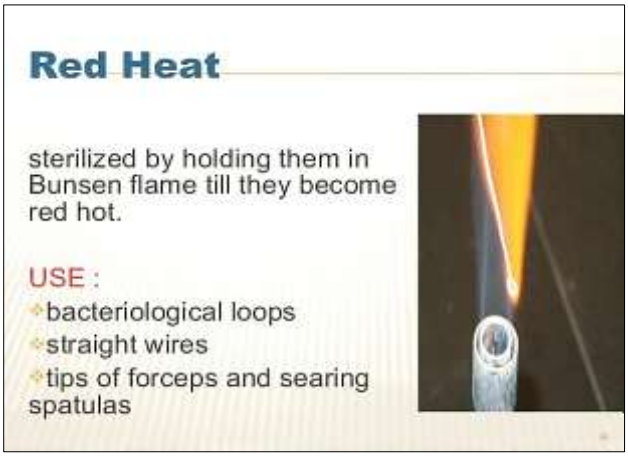




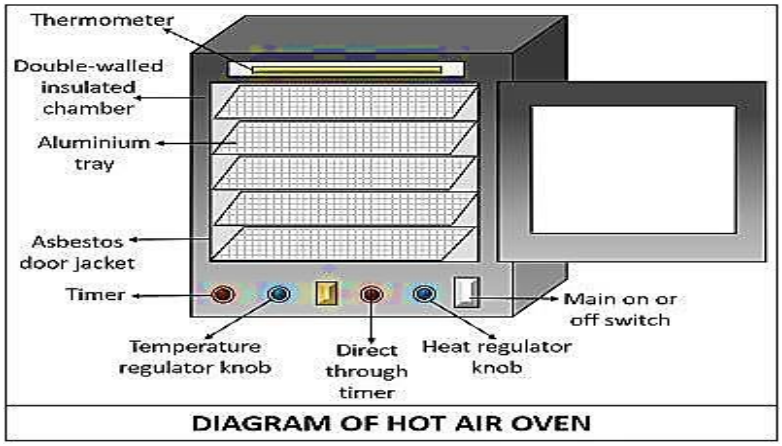
Flowchart 4.1: Physical Methods of Sterilization

Dry Heat Sterilization




Red Heat
sterilized by holding them in Bunsen flame till they become red hot.
USE :
bacteriological loops
straight wires
tips of forceps and searing spatulas

• Dry heat:
2. Flaming: Materials are passed through the flame of a bunsen burner without allowing them to become red hot.
» Glass slides
» scalpels
» Mouths of culture tubes



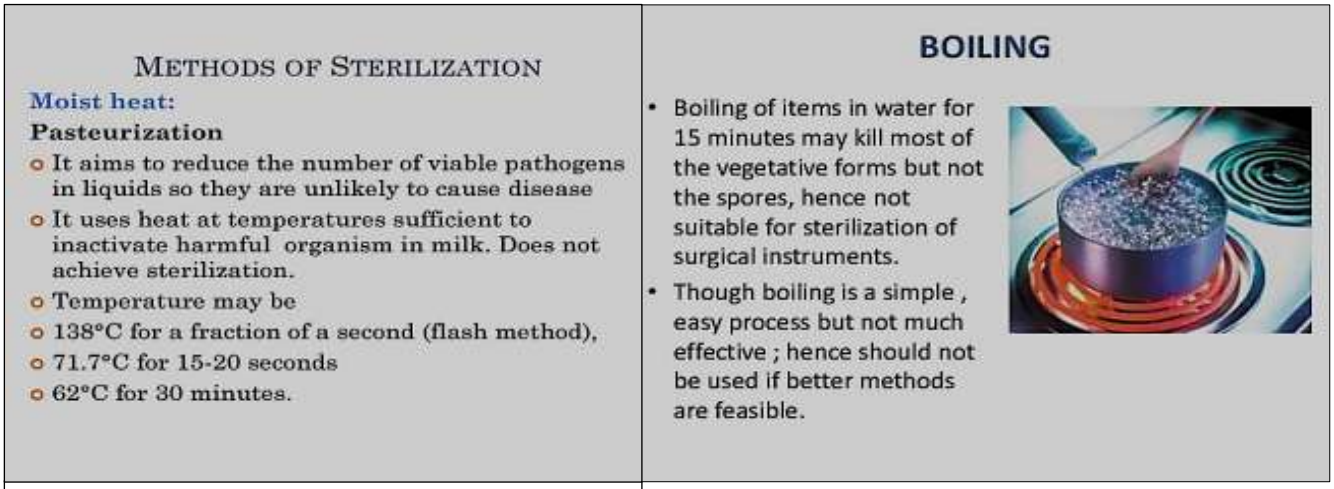
Hot Air Oven

Physical sterilization method in which heat-resistant medical devices are exposed to dry heat. Hot air ovens use extremely high temperatures over several hours to destroy microorganisms and bacterial spores. The ovens use conduction to sterilize items by heating the outside surfaces of the item, which then absorbs the heat and moves it towards the center of the item. To ensure the killing of all microorganisms (bacteria, fungi, viruses, and spores) the temperature has to be at least 180 °C for a time period of at least 30 minutes. Items that are sterilized in a hot air oven include: glassware (like petri dishes, flasks, pipettes, and test tubes), powders (like starch, zinc oxide, and sulfadiazine), materials that contain oils, metal equipment (like scalpels, scissors, and blades)

Moist Heat Sterilization

One of the most common is applying moist heat which includes autoclaving (pressure cooking), boiling, and Tyndallization.
Moist heat destroys microorganisms by the irreversible denaturation of enzymes and structural proteins. Also, Pressure serves as a means to obtain the high temperatures necessary to quickly kill microorganisms. Specific temperatures must be obtained to ensure the microbicidal (***an agent that destroys microbes***) activity.

1. **Temperature below and at 100°C :**


METHODS OF STERILIZATION
Moist heat:
Pasteurization
It aims to reduce the number of viable pathogens in liquids so they are unlikely to cause disease
It uses heat at temperatures sufficient to inactivate harmful organism in milk. Does not achieve sterilization.
Temperature may be
138°C for a fraction of a second (flash method),
71.7°C for 15-20 seconds
62°C for 30 minutes.
BOILING
Boiling of items in water for 15 minutes may kill most of the vegetative forms but not the spores, hence not suitable for sterilization of surgical instruments.
Though boiling is a simple , easy process but not much effective ; hence should not be used if better methods are feasible.

Water bath *delivers sterilized items that need to be either air-dried or manually dried*

2. ***Tyndallization***

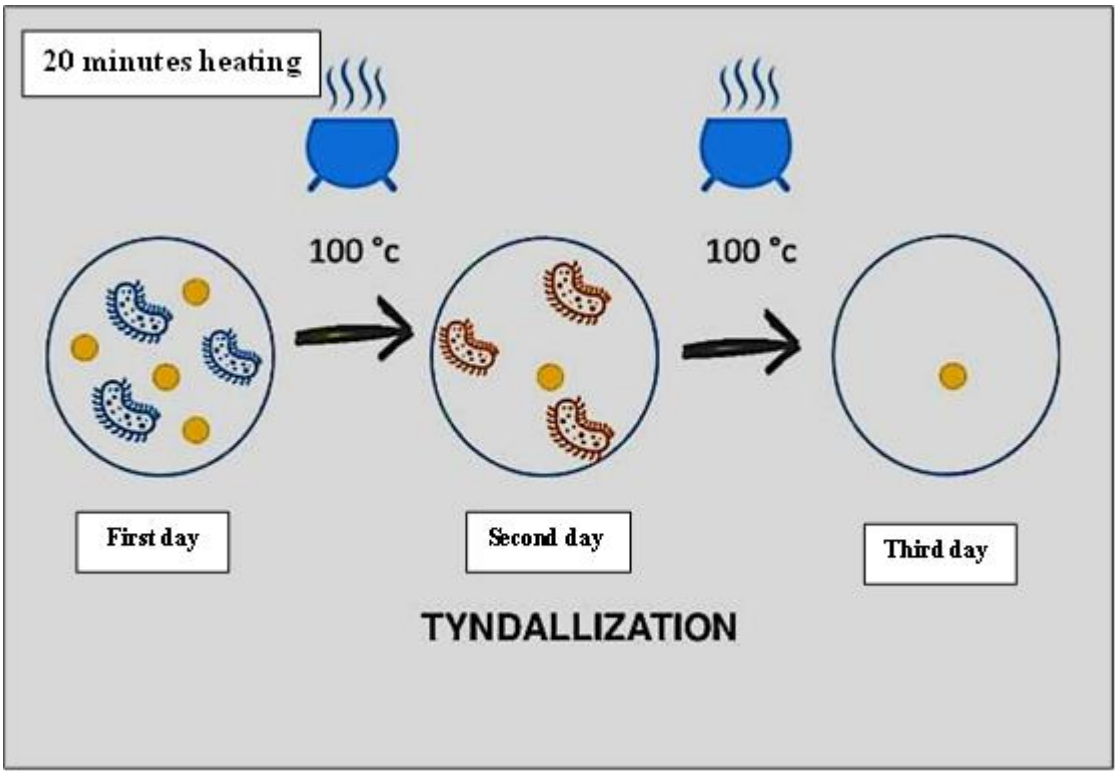
A presentation of steam at 100ºC for 20 minutes on three progressive days is called tyndallization or irregular sanitization. This is a fractional strategy for sterilization. Tyndallization, likewise called fractional sterilization and discontinuous heating, is a type of sanitization. The term sanitization implies the expulsion of every single living microorganism, including spores. The employments of sterilization methods extend from medical instruments to foods. Practically speaking, tyndallization was utilized generally for food stockpiling.

a. ***Principle***

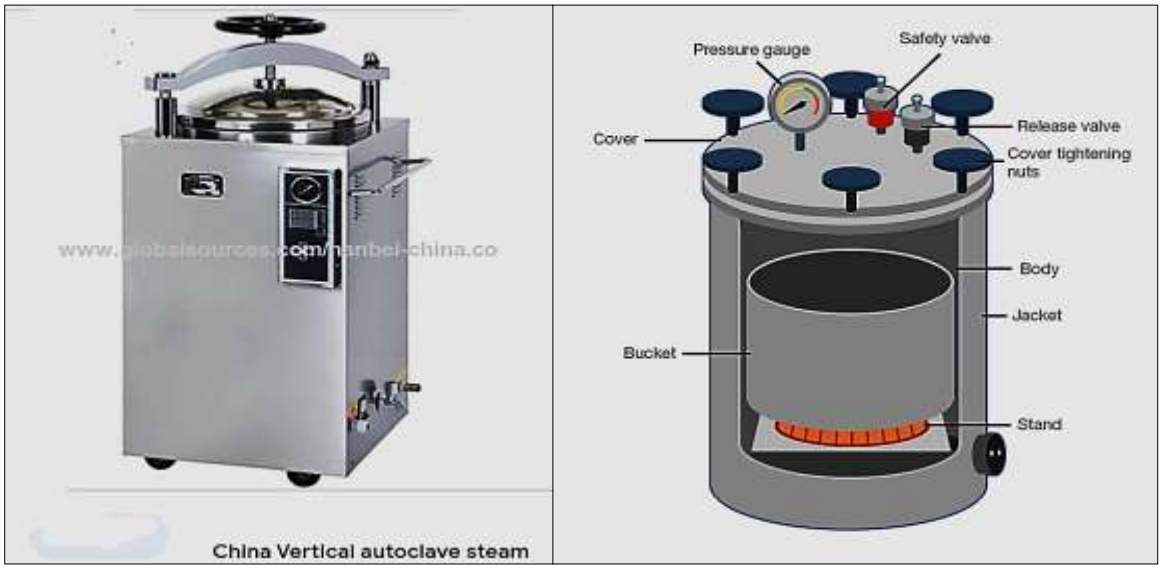
Vegetative cells and a few spores are killed during the first warming and that the more safe spores subsequently develop and are killed amid either the second or the third warming. In spite of the fact that for the most part sufficient, this technique may fall flat with spores of specific anaerobes furthermore, thermophiles.

b. ***Uses***

This technique is helpful in sanitizing heat-sensitive culture media containing such materials as sugars, egg or serum. which are harmed by higher temperature of autoclave.



3. **Principle of Autoclave (Temperature above 100^{o}c)**



Autoclaving is used to sterilize plastic and glass bottles, plastic caps, medical instruments, applicators, and numerous other items that can withstand high temperatures and pressures. Products or components used in the pharmaceutical or medical field require control of microbial levels during processing and handling.

The Importance of the Microbiology Lab for Medical Students(2)

Clinical microbiology laboratory plays an important role in patient care by

- Providing the cause of infection and antimicrobial susceptibility data to physicians.
- Rapid diagnosis of pathogens is important for starting effective antibiotic management and improving the outcomes of treatment.

1. ***What is the importance of studying medical microbiology for a medical student?***

In medical school, microbiology is educated in the context of ***disease pathogenesis.***

Medical microbiology focuses on the prevention and treatment of diseases caused by microorganisms.

Medically-qualified doctors need to complete specialty training in medical microbiology and have the option of training further in infectious diseases and tropical medicine.

2. ***Why is microbiology important to the medical field*?**

Medical microbiologists provide services as follow:

a. Aid the diagnosis and management of infectious diseases.

b. Help ensure the safety of those at risk of acquiring infectious diseases, both in hospitals and the community

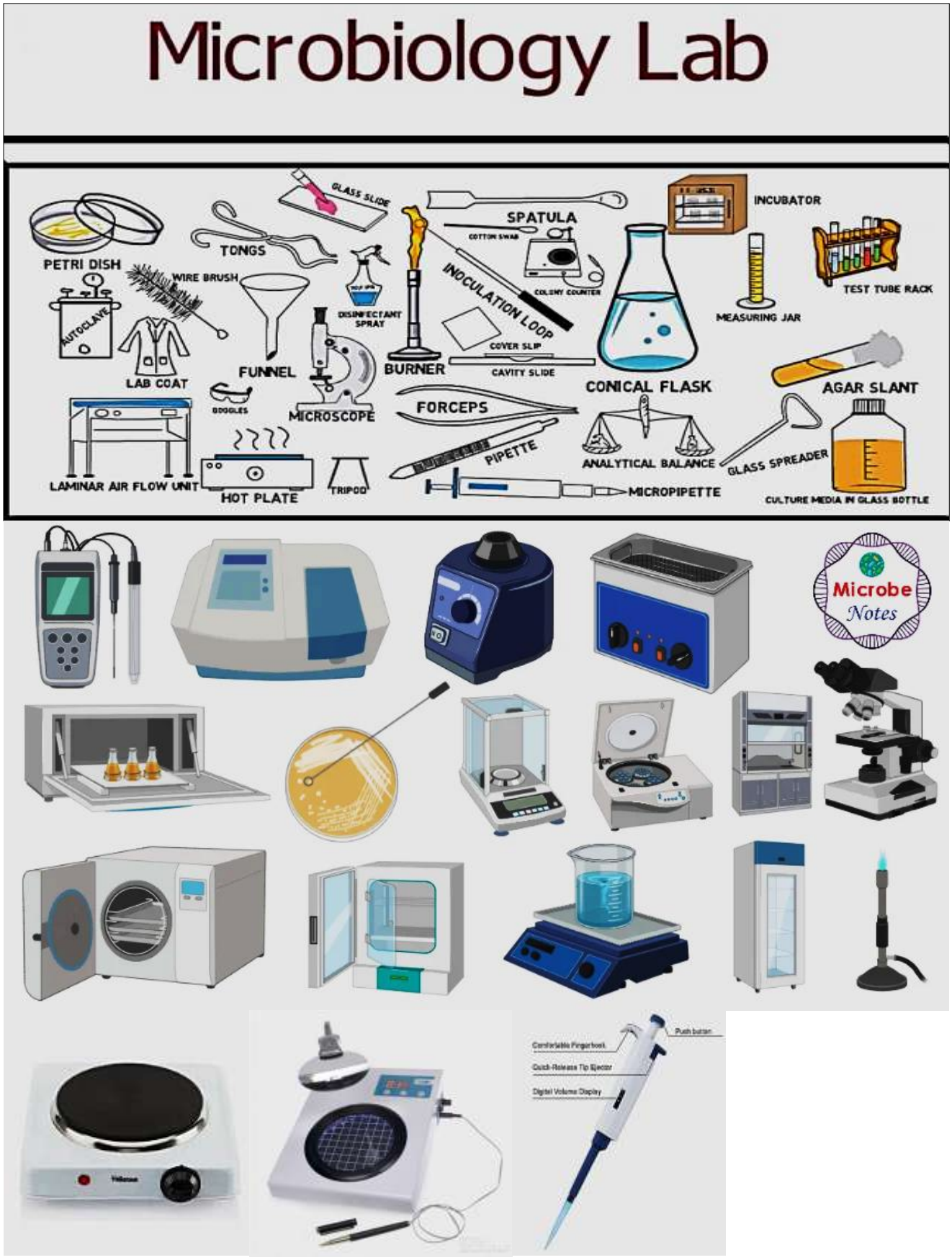
Branches of Microbiology:

1. Bacteriology: the study of bacteria.
2. Immunology: the study of the immune system.
3. Mycology: the study of fungi, such as yeasts and molds.
4. Nematology: the study of nematodes (roundworms).
5. Parasitology: the study of parasites.
6. Phycology: the study of algae.

What are the basic tools used in a microbiology lab?

Microbiology equipment include:

Microscopes; slides; test tubes; petri dishes; growth mediums, both solid and liquid; inoculation loops; pipettes and tips; incubators; autoclaves, laminar flow hoods, and more.


Microbiology Lab
GLASS SLIDE
SPATULA
INCUBATOR
PETRI DISH
TONGS
COTTON SWAB
COLONY COUNTER
WIRE BRUSH
INOCULATION LOOP
TEST TUBE RACK
AUTOCLAVE
DISINFECTANT SPRAY
MEASURING JAR
LAB COAT
FUNNEL
BURNER
COVER SLIP
CAVITY SLIDE
CONICAL FLASK
AGAR SLANT
GOGGLES
MICROSCOPE
FORCEPS
PIPETTE
ANALYTICAL BALANCE
GLASS SPREADER
LAMINAR AIR FLOW UNIT
HOT PLATE
TRIPOD
MICROPIPETTE
CULTURE MEDIA IN GLASS BOTTLE
Microbe Notes

Microbiology Equipment

Note these tools carefully and then record their use in the microbiology lab

(Your homework)

1)Microscopes 2) Slides

3)Test Tubes 4)Petri Dishes

4) Growth Mediums (Solid And Liquid)

5)Inoculation Loops 6) Pipettes

7) Tips 8) Glassware 9)Incubators

10) Autoclave 11) Micropipette

12) Laminar Flow Hood (Cabinet 1)

13) Bunsen Burner 14) pH meter

15) Vortex Mix 16) Colony Counter

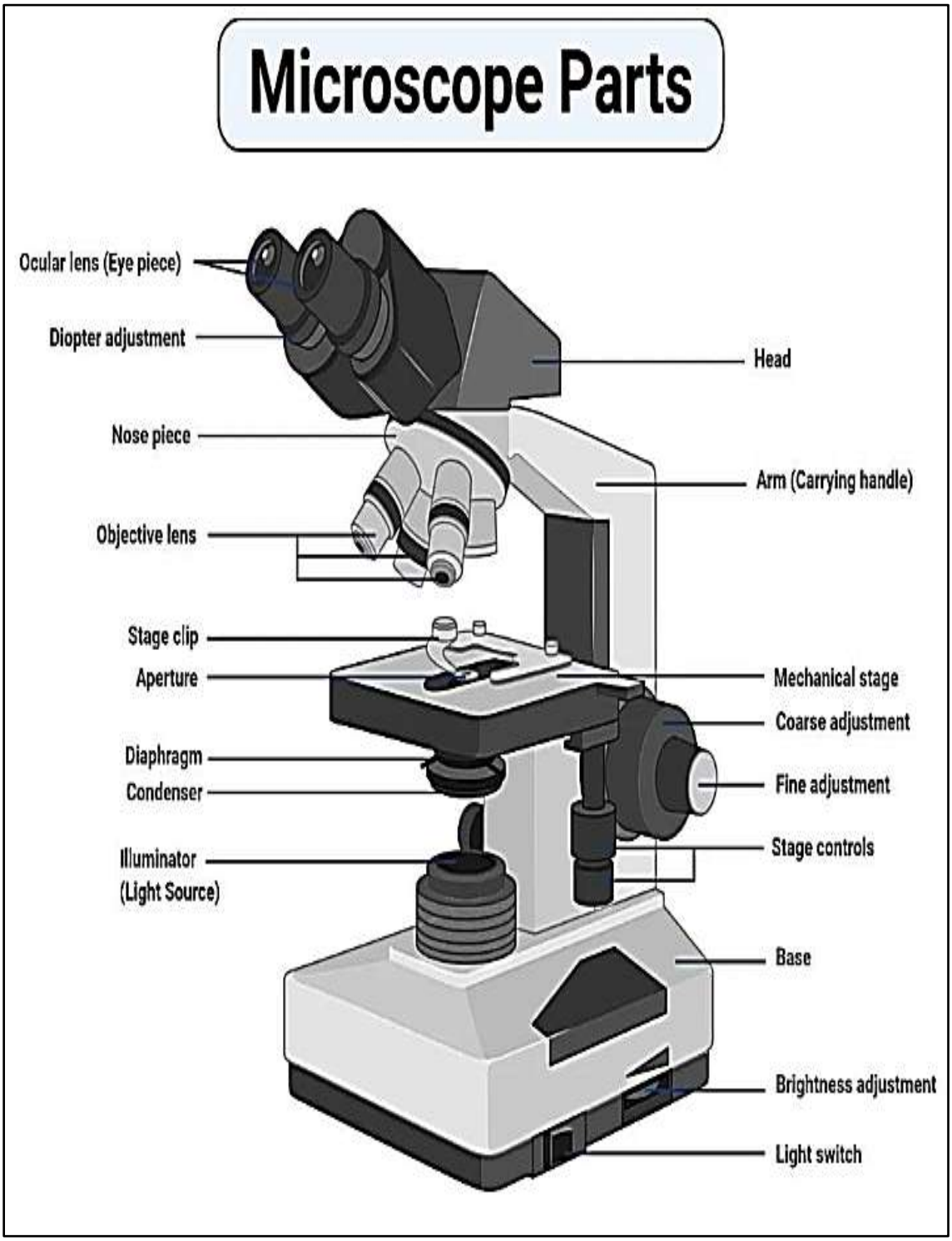
17) Water Bath 18) Centrifuge

19) Hot Air Oven 20) Autoclave

21) Balance 22) Refrigerator

Microscopical Examination of Smears(Lab.3)

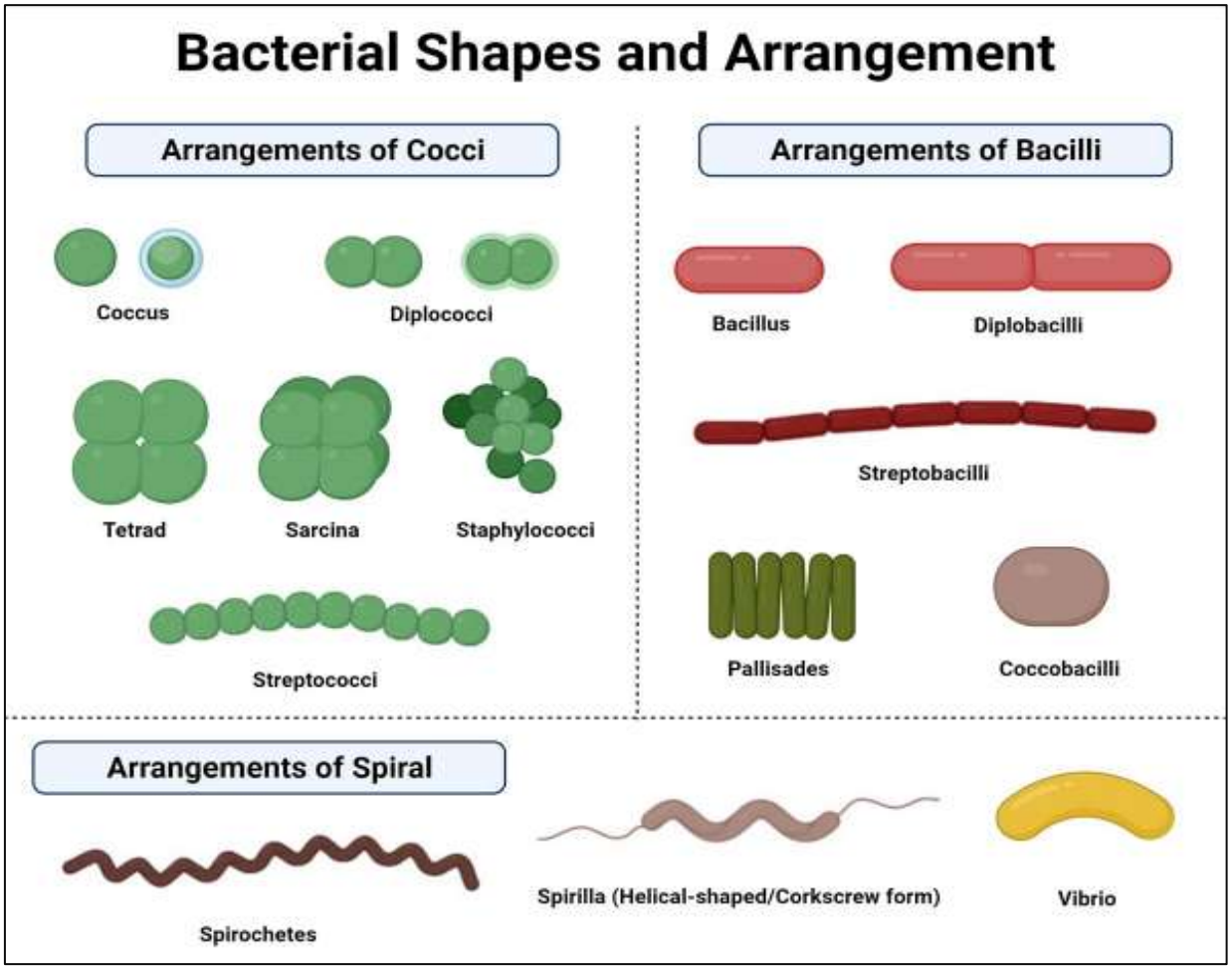
Compound Microscope



Parts of the microscope

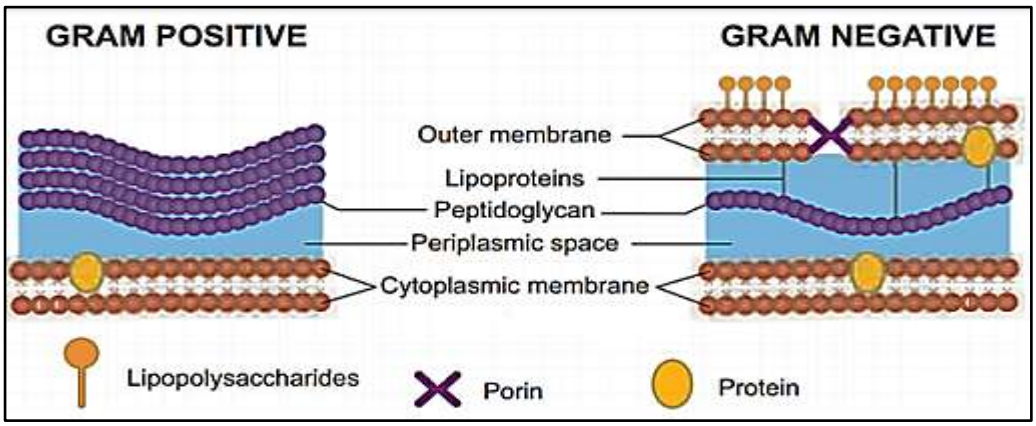
Part of microscope	Function
Eyepiece Lens	Magnifies the specimen
Coarse Adjustment Knob	Used to focus on Low Power
Fine Adjustment Knob	Used to focus on Medium and High Power
Objective Lens	Magnifies the specimen
Nosepiece	Allows the objective lens to be changed
Stage Clips	Holds the slide in place on the stage
Diaphragm	Controls amount of light entering condenser
Condenser	Concentrates light into a beam
Light Source	Projects light upwards through microscope

Classification Bacteria: according to Shape / Gram Stain



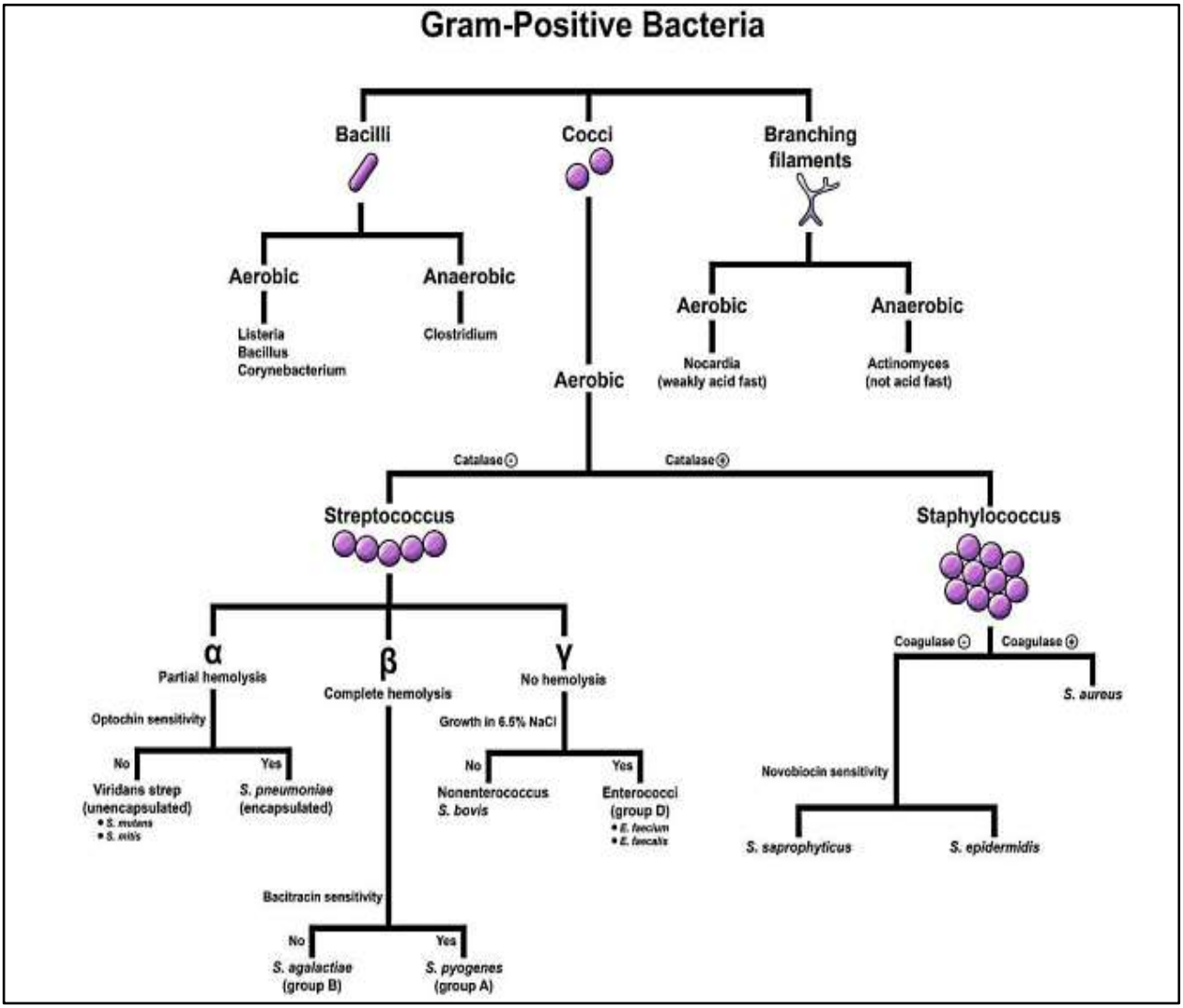
1. Gram-Staining

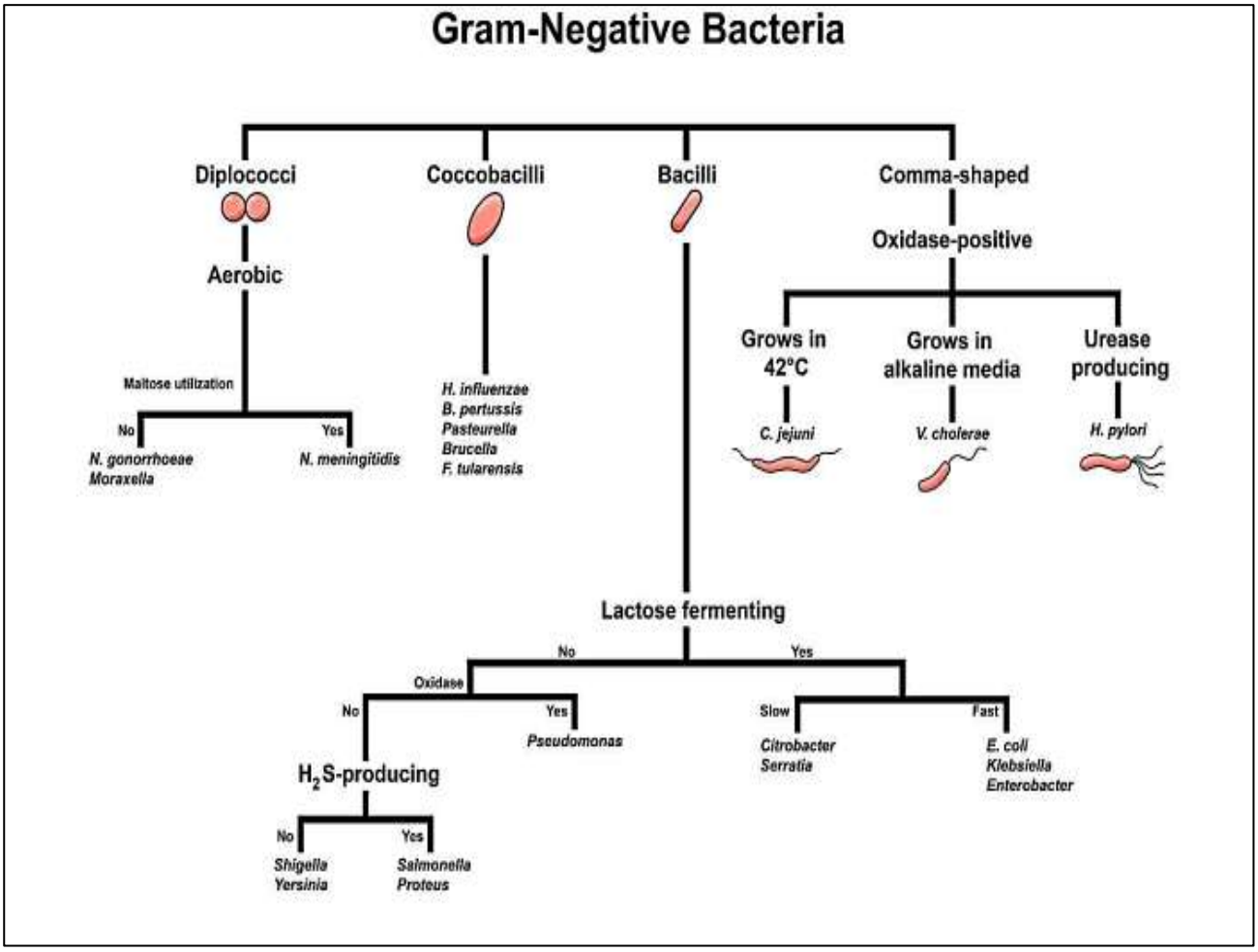
The Gram stain procedure distinguishes between Gram positive and Gram negative groups by coloring these cells red or violet. Gram positive bacteria stain violet due to the presence of **a thick layer of peptidoglycan in their cell walls**, which retains the crystal violet these cells are stained with



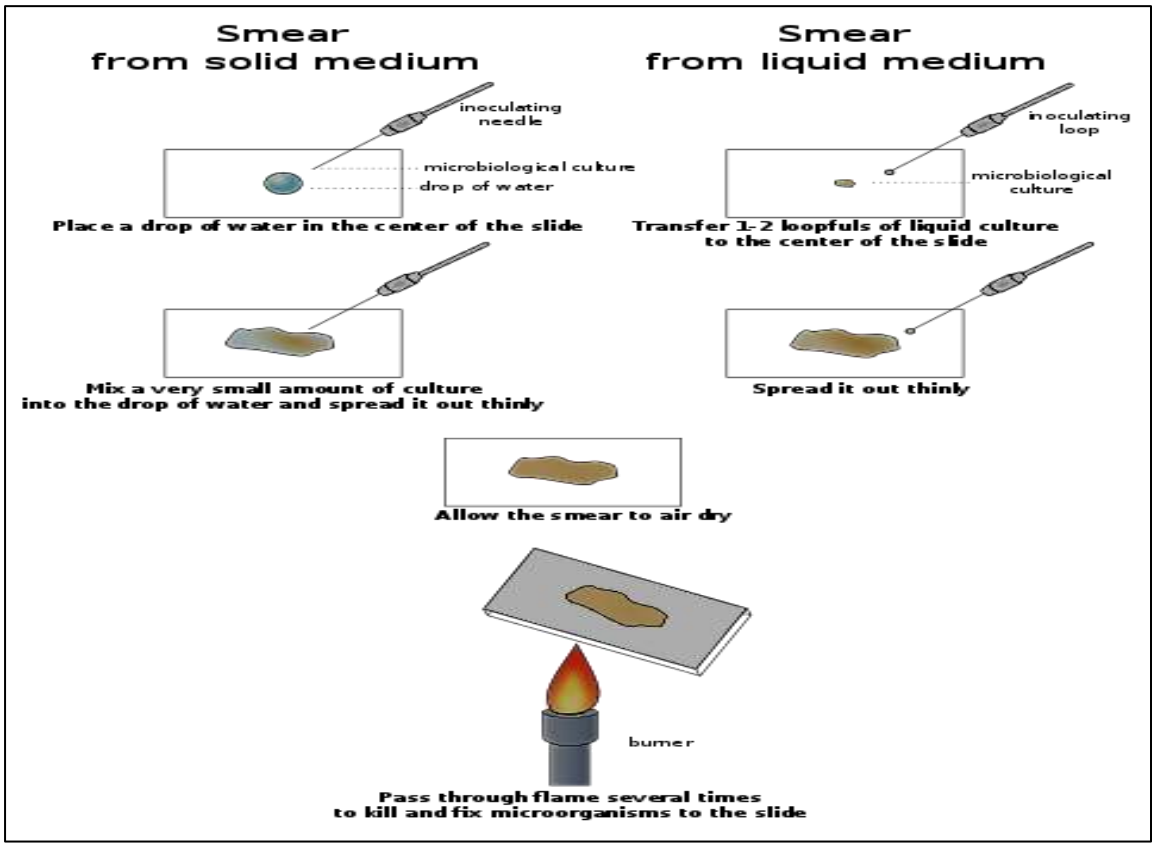
Classification bacteria according to Gram-Staining

(Gram-positive and Gram-negative)





Technique of preparing bacterial smear


Smear from solid medium
inoculating needle
microbiological culture
drop of water
Place a drop of water in the center of the slide
Mix a very small amount of culture into the drop of water and spread it out thinly
Smear from liquid medium
inoculating loop
microbiological culture
Transfer 1-2 loopfuls of liquid culture to the center of the slide
Spread it out thinly
Allow the smear to air dry
burner
Pass through flame several times to kill and fix microorganisms to the slide

- **Gram Stain**
  - Most species of bacteria are classified into two categories based on the structure of their cell walls as determined by a technique called the **Gram stain.**
  - **Gram-positive bacteria** have a thick layer of peptidoglycan in their cell wall, and they appear violet under a microscope after the Gram-staining procedure.
  - **Gram-negative bacteria** have a thin layer of peptidoglycan in their cell wall, and they appear reddish-pink under a microscope after the Gram-staining procedure.

Gram stain ingredients

- Crystal violet (primary stain)
- Iodine solution/Gram's Iodine (mordant that fixes crystal violet to cell wall)
- Decolorizer (e.g. ethanol)
- Safranin (secondary stain)
- Water (preferably in a squirt bottle)

Steps for Gram Staining

Step 1:
Flood heat-fixed emulsion with Crystal Violet (primary stain), let stand for 1 minute, rinse with water
Step 2:
Add iodine solution, let stand for 1 minute, rinse with water
Step 3:
Decolorize with alcohol solution holding slide on slight angle, add until run off is clear (20-30 sec), rinse with water
Step 4:
Counter-stain with Safranin, let stand for 1 minute, rinse with water

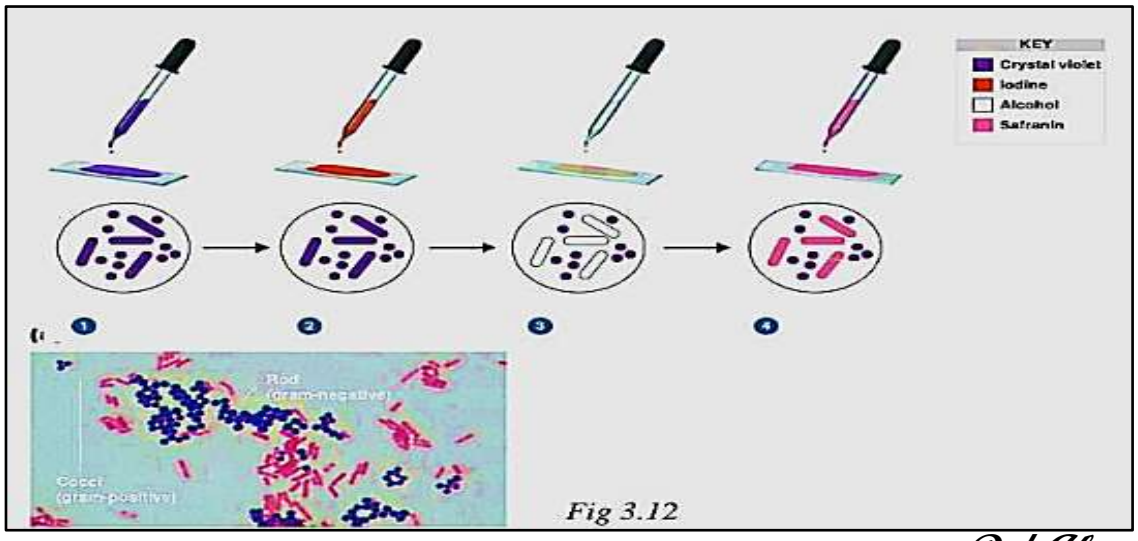


Fig 3.12

2. Ziehl–Neelsen stain:

The most common staining technique used to identify acid-fast bacteria is the Ziehl–Neelsen stain, in which the acid-fast species are stained ***bright red*** and ***stand out*** clearly against ***a blue background***. Bacteria displaying acid fastness include: Genus Mycobacterium – M. leprae, M. tuberculosis, and *Nocardia*

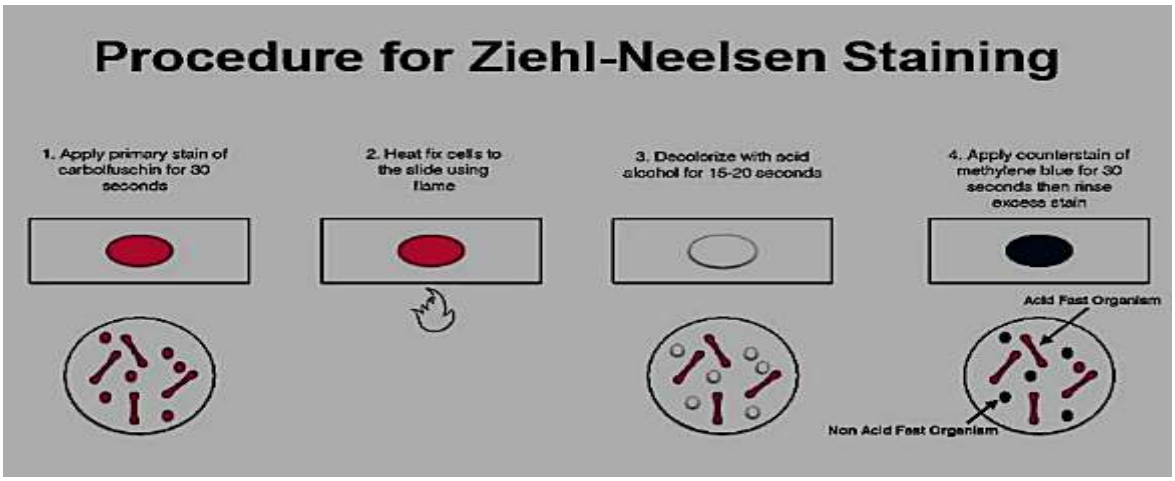
3-8: Acid Fast Stain (Acid Fast Bacillus (AFB)
Purpose: to stain acid fast bacterial cell walls and differentiate acid-fast bacteria from non-acid-fast bacteria.
Mycobacterium- has a cell wall made of 60% mycolic acid Adherence factor
AF (-)
AF (+)

Ziehl–Neelsen stain ingredients

a. primary stain (strong/concentrated Carbol fuchsin),
b. Decolorizer (20% H2SO4) and
c. counterstain (Loffler's methylene blue)**.**

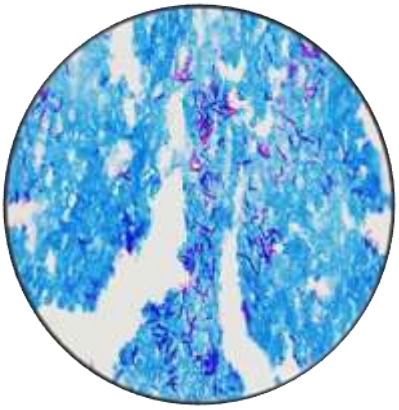
Following are the 4 step in acid fast staining.

1. First, cover the smear on the slide with Carbol fuchsin and start heating until steam rises. Allow the smear + stain for 5 minutes. After 5 minutes wash the slide.
2. Cover the slide with 20% Sulphuric acid (H_2SO_4). The red color of the smear change into a yellowish-brown.
3. Wash the slide. Repeat step 2. Until the film is only faintly pink in color.
4. Counterstain with methylene blue for 20 to 25 seconds. Wash the slide, dry in the air.

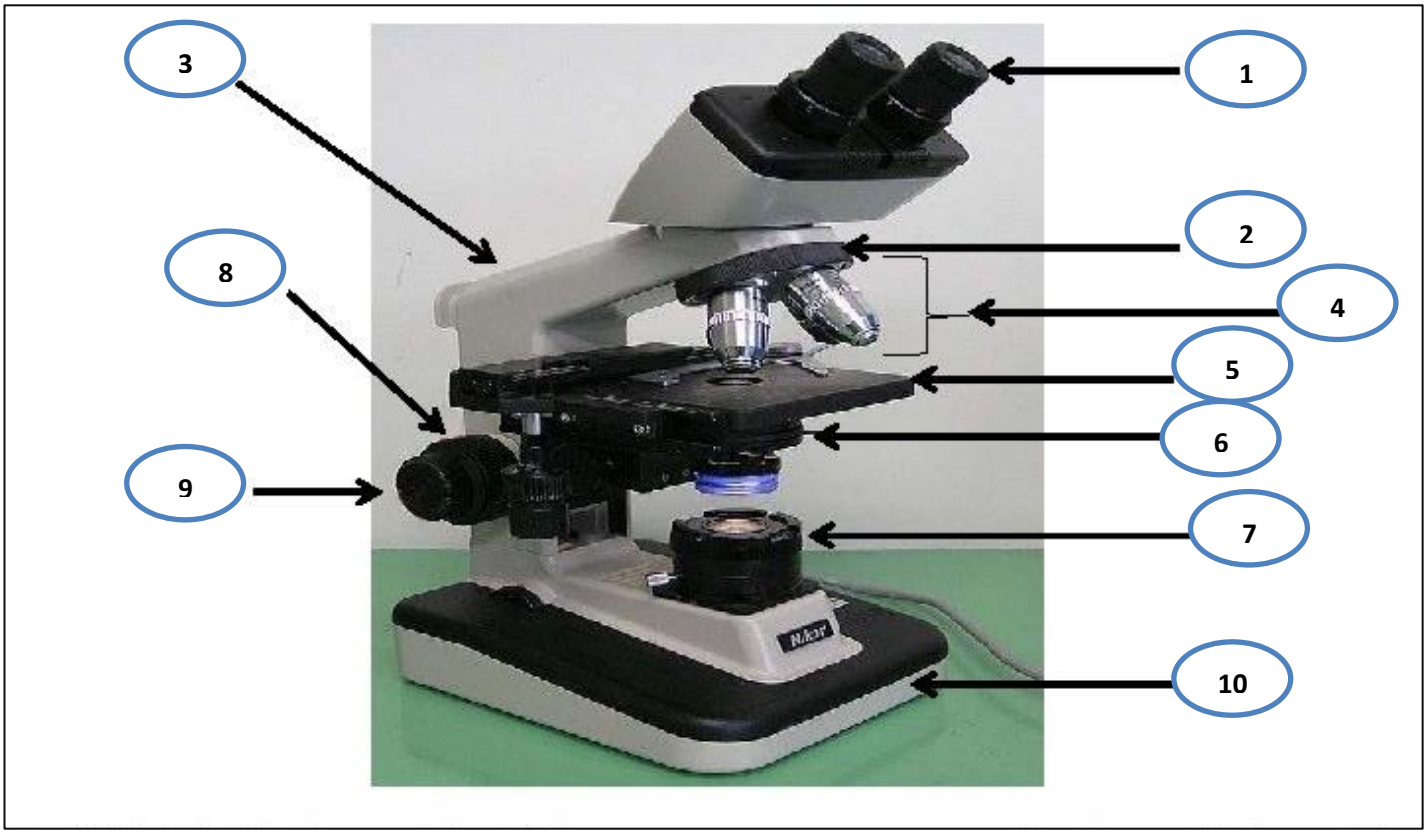

Procedure for Ziehl-Neelsen Staining
1. Apply primary stain of carbolfuschin for 30 seconds
2. Heat fix cells to the slide using flame
3. Decolorize with acid alcohol for 15-20 seconds
4. Apply counterstain of methylene blue for 30 seconds then rinse excess stain
Acid Fast Organism
Non Acid Fast Organism

Summary of Acid-Fast Stain *(Ziehl-Neelsen staining)*

Application of	Reagent	Cell colour	
		Acid fast	Non-acid fast
Primary dye	Carbol fuchsin	Red	Red
Decolorizer	Acid alcohol	Red	Colorless
Counter stain	Methylene blue	Red	Blue

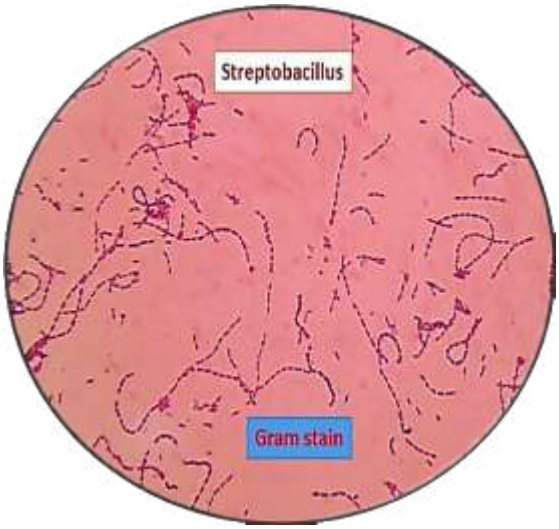


1. **Study this equipment and name its parts.**



2. **Study this slide and determine the type of gram stain bacteria.**

( ) ( )

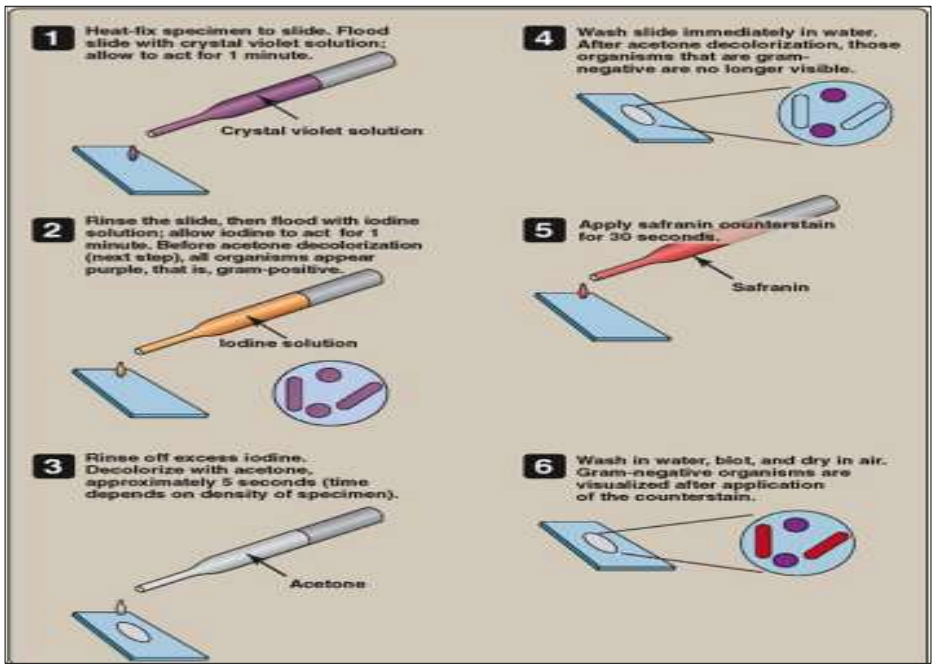


3. Study this picture carefully and answer .

a. Name this technique, _____

b. The aim for using it_____

c. Its component_____


1
Heat-fix specimen to slide. Flood slide with crystal violet solution; allow to act for 1 minute.
Crystal violet solution
2
Rinse the slide, then flood with iodine solution; allow iodine to act for 1 minute. Before acetone decolorization (next step), all organisms appear purple, that is, gram-positive.
Iodine solution
3
Rinse off excess iodine. Decolorize with acetone, approximately 5 seconds (time depends on density of specimen).
Acetone
4
Wash slide immediately in water. After acetone decolorization, those organisms that are gram-negative are no longer visible.
5
Apply safranin counterstain for 30 seconds.
Safranin
6
Wash in water, blot, and dry in air. Gram-negative organisms are visualized after application of the counterstain.

4. Study this picture carefully and answer

a. In which technique these stains are used._____

b. The aim for using it._____

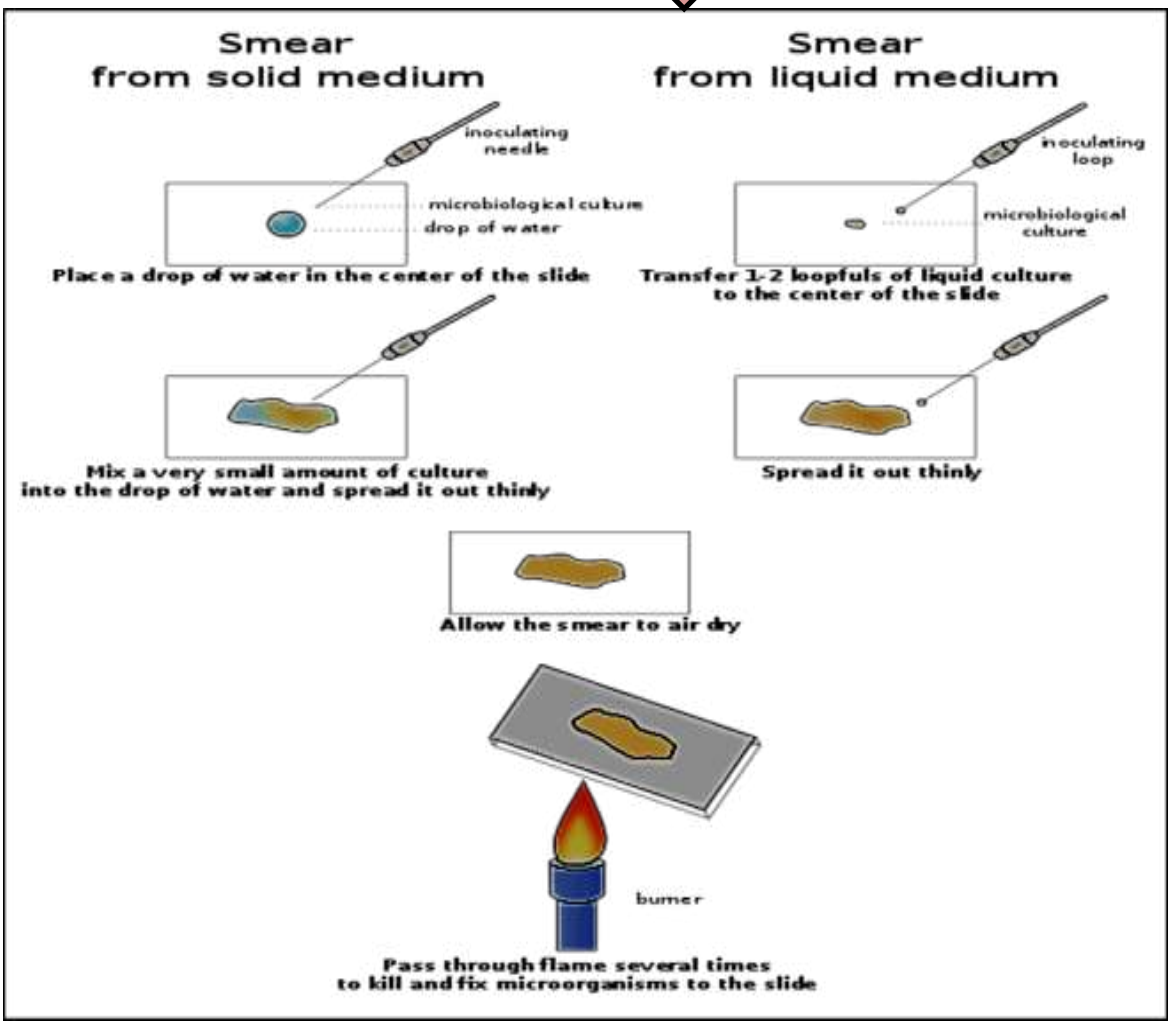
c. determine the name of bacteria _____

Practical Staining Techniques in Microbiological Lab(4)

Stains are used to recognize bacteria in clinical specimens and in cultures. There are a lot of types of these stains. In this lab. You focus on two stains as follow:

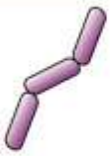
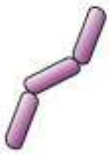
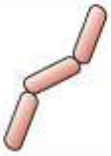
1. ***Gram stain***: it is one of the most useful tools in the microbiology laboratory and is used universally. Gram-stained smears are read promptly to determine the relative numbers and morphology of bacteria and cellular structures in the specimen.
2. ***Acid Fast Stain*** (Ziehl-Neelsen stain): Members of the bacterial genus *Mycobacterium* contain large amount of lipids (fatty) substances within their cell walls. These fatty waxes resist staining by ordinary methods. For that applying heat lead to staining the cells by penetrate the lipid cell wall and reach the cell cytoplasm.

→ First of all, a smear must be prepared for the microbe that you want to know its morphological characteristics as figure below


Smear
from solid medium
inoculating needle
microbiological culture
drop of water
Place a drop of water in the center of the slide
Mix a very small amount of culture into the drop of water and spread it out thinly
Smear
from liquid medium
inoculating loop
microbiological culture
Transfer 1-2 loopfuls of liquid culture to the center of the slide
Spread it out thinly
Allow the smear to air dry
burner
Pass through flame several times to kill and fix microorganisms to the slide

Smear of bacteria

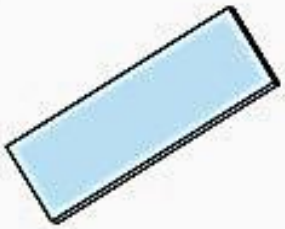
1. Gram stain ingredients

Gram stain process			
Gram staining steps	**Cell effects**	**Gram-positive**	**Gram-negative**
Step 1 **Crystal violet** *primary stain added to specimen smear.*	Stains cells purple or blue.		
Step 2 **Iodine** *mordant makes dye less soluble so it adheres to cell walls.*	Cells remain purple or blue.		
Step 3 **Alcohol** *decolorizer washes away stain from gram-negative cell walls.*	Gram-positive cells remain purple or blue. Gram-negative cells are colorless.		
Step 4 **Safranin** *counterstain allows dye adherance to gram-negative cells.*	Gram-positive cells remain purple or blue. Gram-negative cells appear pink or red.		

GRAM STAINING

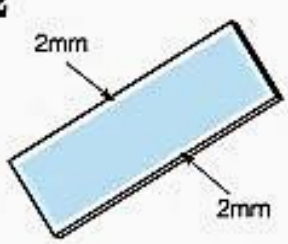
Flow Through Procedure

1



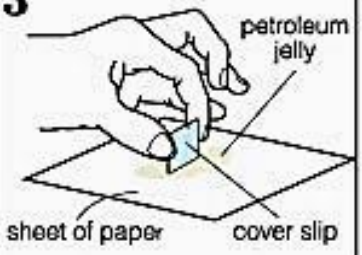
Wipe bottom of biofilm slide clean

2



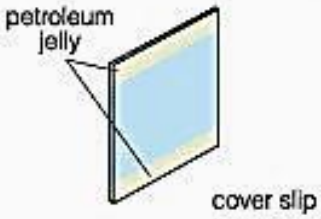
Clean top edges of slide about 2mm

3



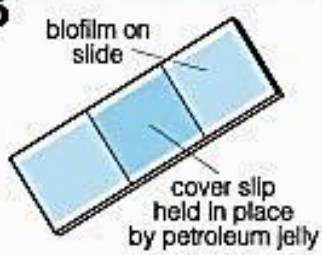
Build up a ridge of petroleum jelly on the top and bottom of a cover slip

4



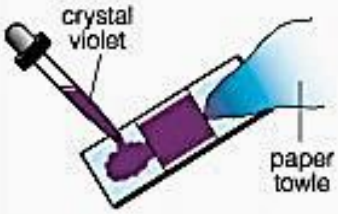
Cover slip with petroleum jelly

5



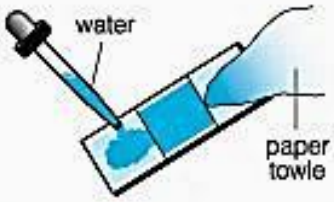
Biofilm on slide with cover slip

6



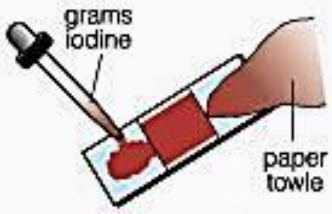
Add crystal violet-wait 30 sec.

7



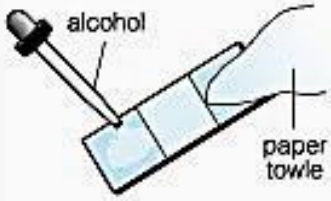
Wash with water

8



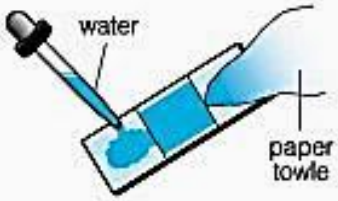
Add Grams Iodine -wait 1.5 min.

9



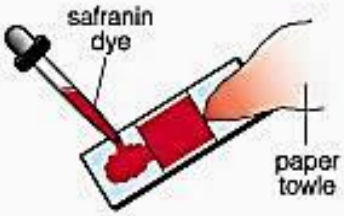
Decolorize with alcohol

10



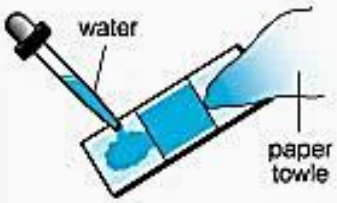
Wash with water

11



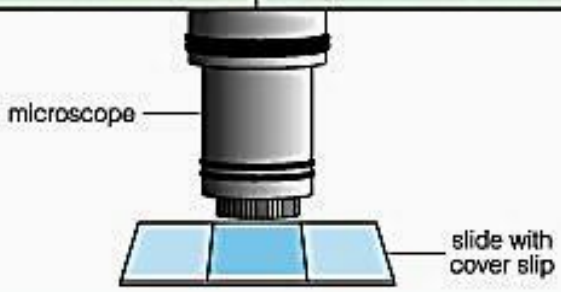
Stain with Safranin dye-wait 30 sec.

12

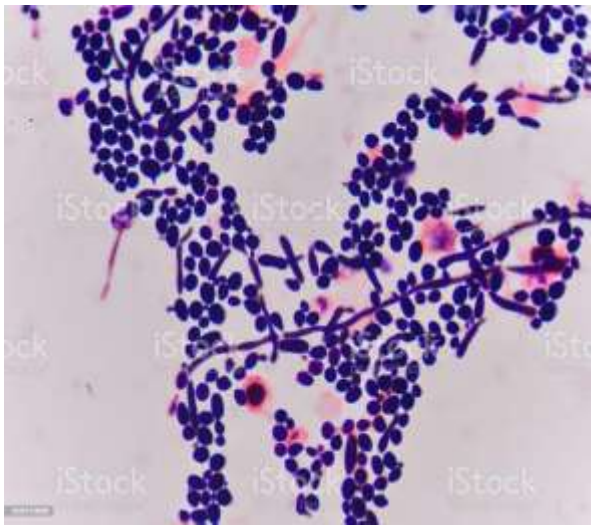


Wash with water

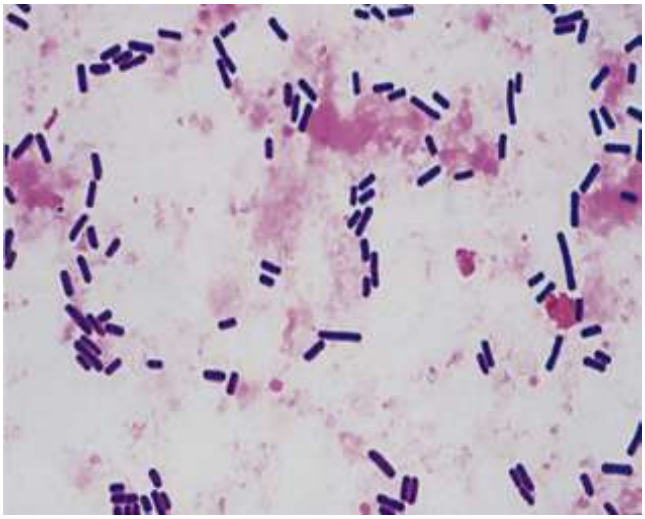
13



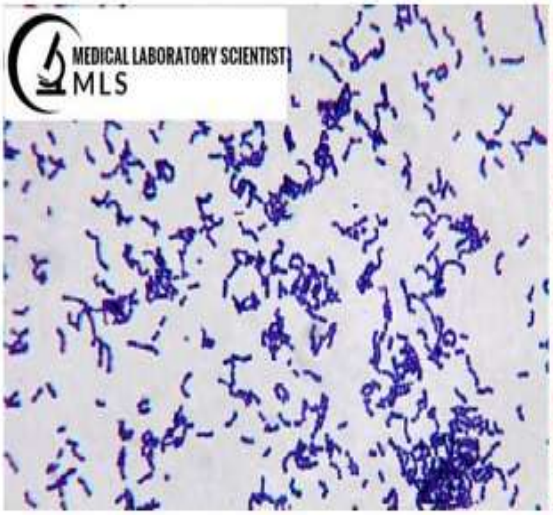
Examine under oil immersion through the cover slip



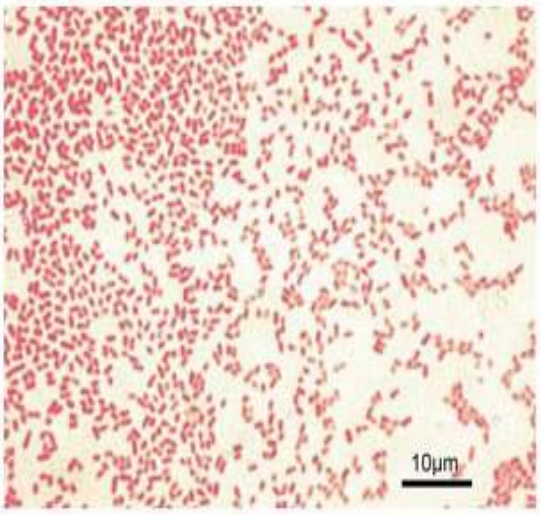
Yeast



Gram Positive Bacilli

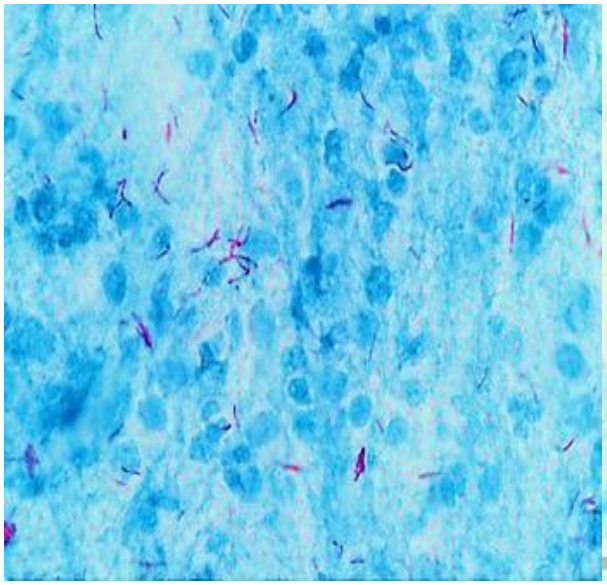


Gram Positive Bacteria

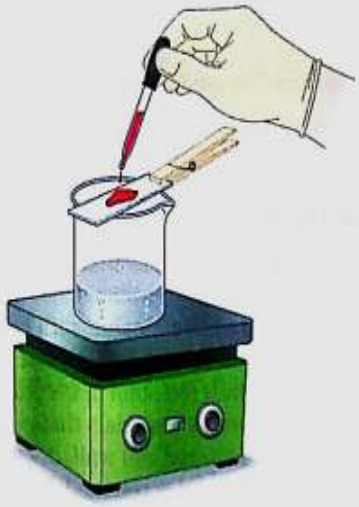


Gram Negative Bacteria

Ziehl-Neelsen staining ingredients



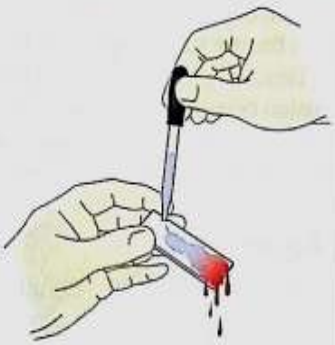
PROCEDURE



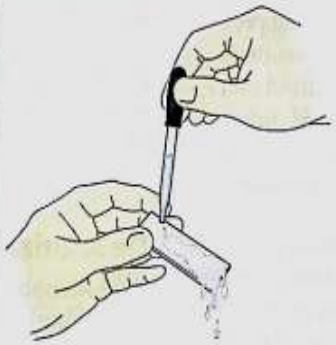
or

1a **Heat method:** Apply carbol fuchsin and steam over a beaker of boiling water that is placed on a hot plate for 5 minutes. Do not allow the stain to evaporate.

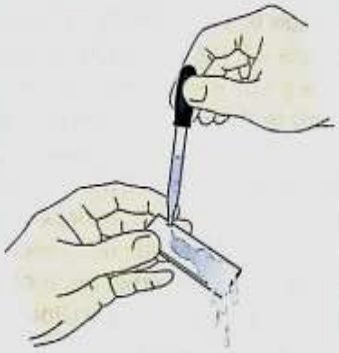
1b **Heatless method:** Apply carbol fuchsin with Tergitol for 5 to 10 minutes.



2 Cool and wash off stain with tap water.

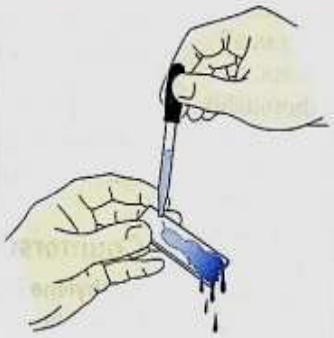


3 Add acid-alcohol drop by drop until the alcohol runs almost clear.

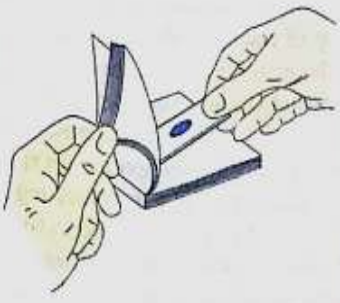


4 Wash off the acid-alcohol with tap water.

5 Counterstain with methylene blue for 2 minutes.



6 Wash off the methylene blue with tap water.



7 Blot the slide dry with bibulous paper.

Figure 10.1 Acid-fast staining procedure

Types of Culture Media Used in Microbiology(5)

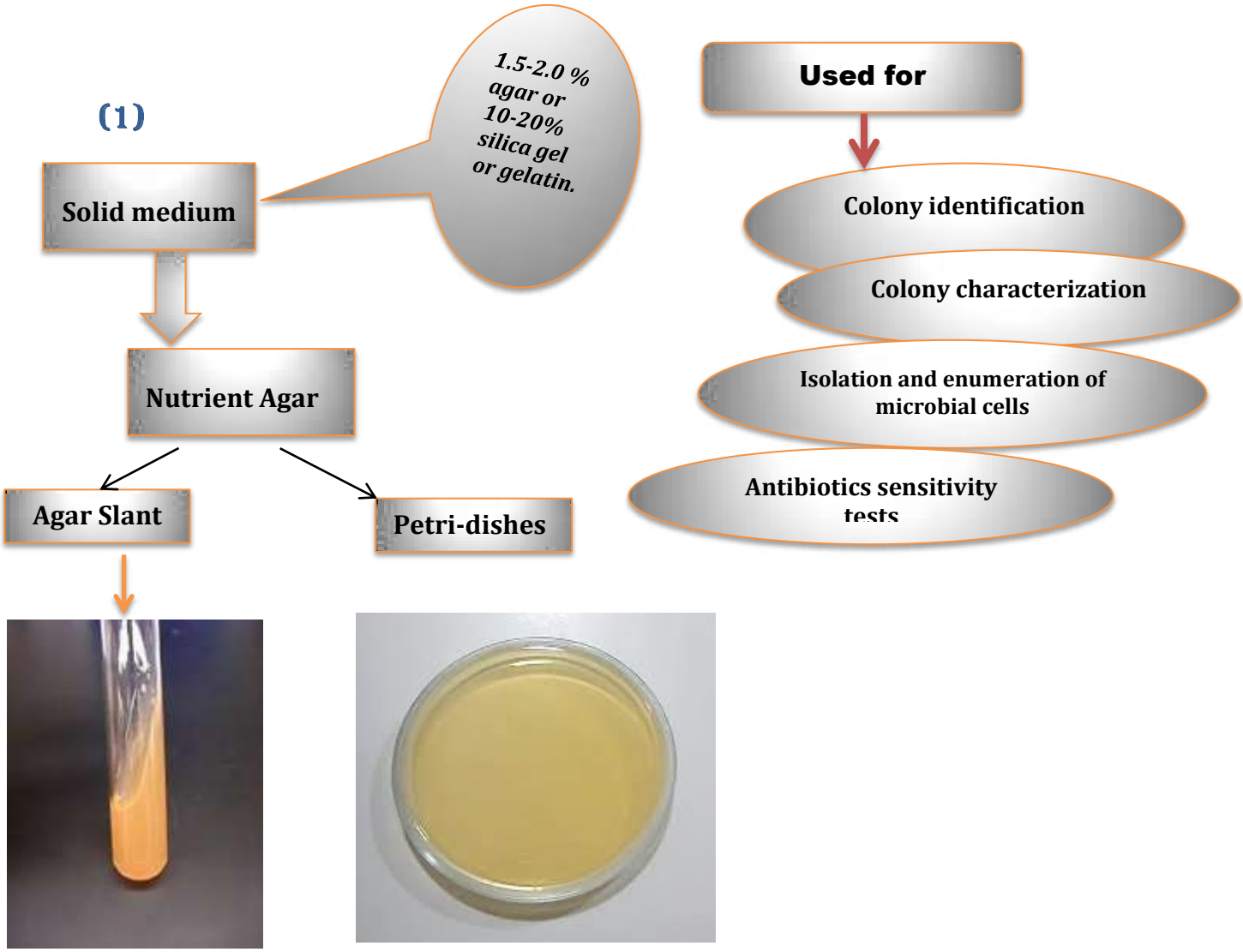
Most medically important bacteria and fungi can be cultured on laboratory media.

What is bacterial culture media?

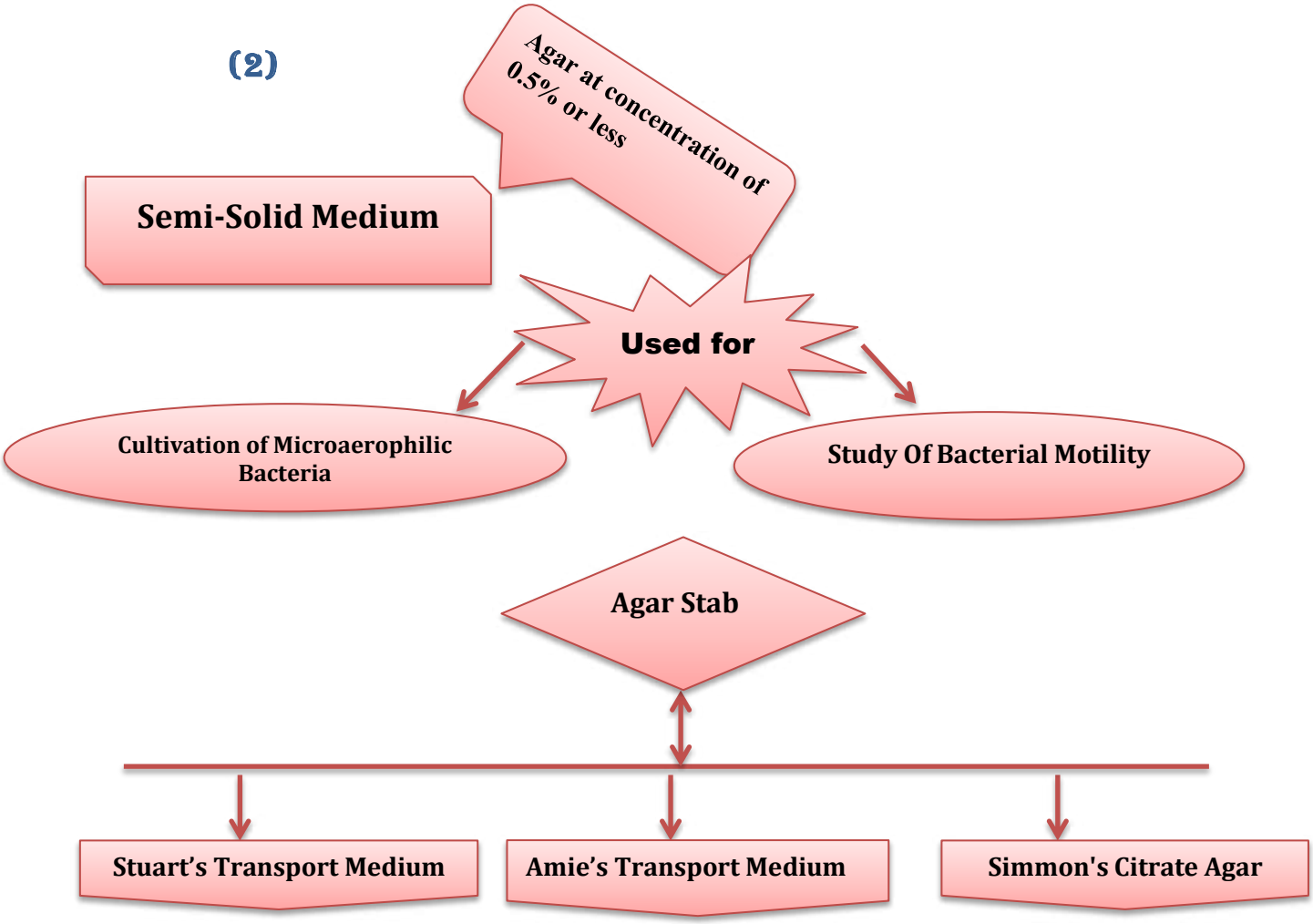
It is the nutrient preparation or a mixture of substances that promotes and supports the growth and differentiation of microorganisms.

Bacterial culture media can classified on the basis of three types:

a. ***Classification of bacterial Culture media on the basis of <u>Consistency</u>***



(2)


Agar at concentration of 0.5% or less
Semi-Solid Medium
Used for
Cultivation of Microaerophilic Bacteria
Study Of Bacterial Motility
Agar Stab
Stuart's Transport Medium
Amie's Transport Medium
Simmon's Citrate Agar

A
B

(3)

Liquid (Broth) Medium → **Propagation of A Large Number of Microbes.**

Fermentation Studies

Biochemical Test

without have any trace of gelling agents

Bottles and tubes

- ***Brilliant Green Bile Broth***
- ***MacConkey broth***
- ***Peptone Water***
- **Nutrient broth**

b. Classification of bacterial Culture media on the basis of purpose/ functional use/ application

Many special-purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria. To meet these needs, numerous media are available. Some of types of media are used for more than one purpose and are listed under more than one category.

1. General-Purpose Media (basic):

- Basically simple media.
- Support the growth of most non-fastidious bacteria.
- Used for the primary isolation of microorganisms.

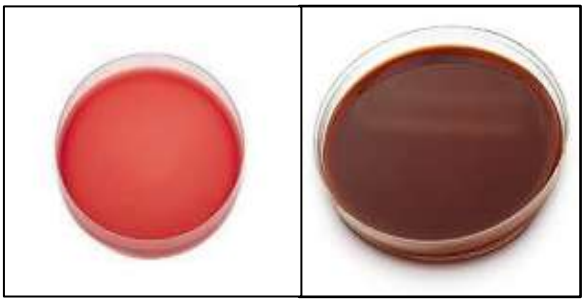
E.x. **Peptone water**, **Nutrient Broth (NB), and Nutrient Agar (NA)**

2. Enriched Media

Contain specific growth substances such as **blood, serum, and egg** with the **nutrient agar** to satisfy the nutritional stresses of metabolically fastidious microorganisms

Some examples :

- **Blood Agar.**
- **Chocolate Agar.**
- **Loeffer's Serum.**



Blood Agar __ Chocolate Agar

3. Enrichment Media

Are liquid in consistency .i.e. it is typically used as a broth

- Designed to inhibit unwanted commensal or contaminating bacteria in the clinical specimen
- Help to recover pathogens from a mixture of bacteria

- Selenite F broth (SFB)
- Alkaline peptone water (APW)
- Thioglycollate broth (THIO)

Selenite F broth

Alkaline peptone water

Thioglycollate broth (THIO)

4. Selective Media

- Inhibits the growth of un desired microorganisms.
- Grants and encourages the growth of desired microorganisms to develop.

It includes addition of antibiotics, dyes, chemicals, alteration of pH, or a combination of these.

Some examples
Deoxycholate-Citrate-Agar (DCA)
Salmonella Shigella agar (SSA)
Mannitol-Salt-broth and agar.(MSB) &(MSA)
Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS)
MacConkey's Agar(MaCC)
Xylose Lysine Desoxycholate (XLD)

DCA

MaCC

TCBS

XLD

SSA

MSB

MSA

5. Differential Medium (Indicator Media)

Are designed in such a way that different bacteria can be recognized on the basis of their colony color

MacCbP

Include incorporation of dyes, metabolic substrates

BGBS

Some examples

- Blood-Agar.(**BA**)
- MacConkey-Agar(**MaCC)**
- Cystine Lactose Electrolyte Deficient **(CLED**) agar.
- Brilliant Green Bile Broth(BGBS)
- MacConkey Broth with bromocresol purple (MacCbP)

CLED

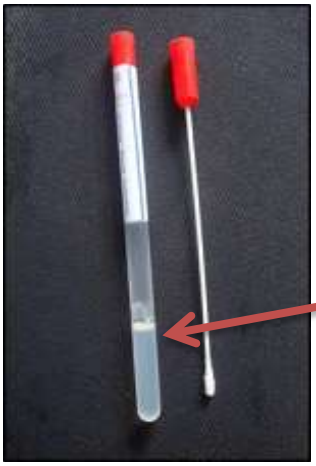
6. Transport Medium

Purpose

1. Inhibit the overgrowth of unwanted bacteria (contaminating organisms or commensals)
2. Maintain the possibility of the potential pathogens.
3. Prevent drying of a specimen.

Some Examples

1. **Amie's transport medium**
2. **Stuart's Transport Medium**



Stuart's Transport Medium

Amie's transport medium

7. Assay Medium

Designated composition are utilized for the assay of vitamins, amino acids, and antibiotics.

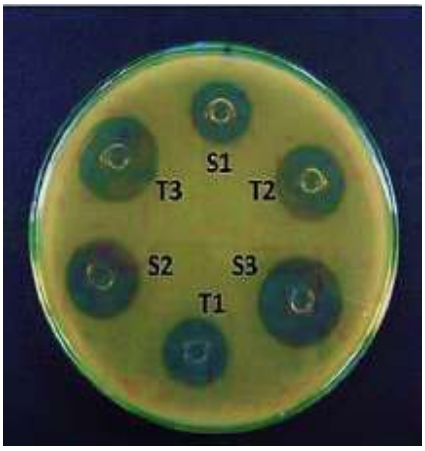
In this medium, the rate of growth is directly proportional to the nutrient concentration

Antibiotic assay media are used for determining antibiotic potency by the microbiological assay technique

Other types of medium include;

a. **Media for enumeration of bacteria,**
b. **Media for characterization of bacteria,**
c. **Maintenance media etc.**

Blood Agar



Nutrient Agar

8. Anaerobic Media

This type of Media should to be enriched by nutrients such as hemin and vitamin K.

Boiling the medium helps to release any suspended oxygen.

Before applying the medium needs to be sterilized in a water bath to dismiss any suspended

Some Examples

- **Robertson Cooked Meat (RCM) medium**
- **Thioglycollate broth**

Robertson Cooked Meat (RCM) medium

Thioglycollate broth

Culture Media Preparation(lab.6)

1. Culture media preparation must be carried out exactly to ensure microbiological growth is correctly promoted.
2. The individual ingredients of the culture media (powders, gels and liquids) must be carefully weighed out according to the culture media formulation recipe.
3. Preparation of culture media formulations, including liquid growth media and culture media based on nutrient agar, is a common procedure in any microbiology laboratory.

What is culture preparation?

Culture media preparation is one of the routine tasks common to many microbiology laboratories.

- Media preparation products provide an optimum growing environment for culturing microbiological organisms.
- Media preparation products differ based on ***the types of nutrients*** and ***their combination***.

What are the ingredients commonly used in the preparation of culture media?

Some of the ingredients of culture media include: water, agar, peptone, casein hydrolysate, meat extract, yeast extract and malt extract.

Classification: Bacterial culture media can be classified in at least three ways:

1. ***Based on consistency.***
2. Based on nutritional component.
3. ***Based on its functional use.***

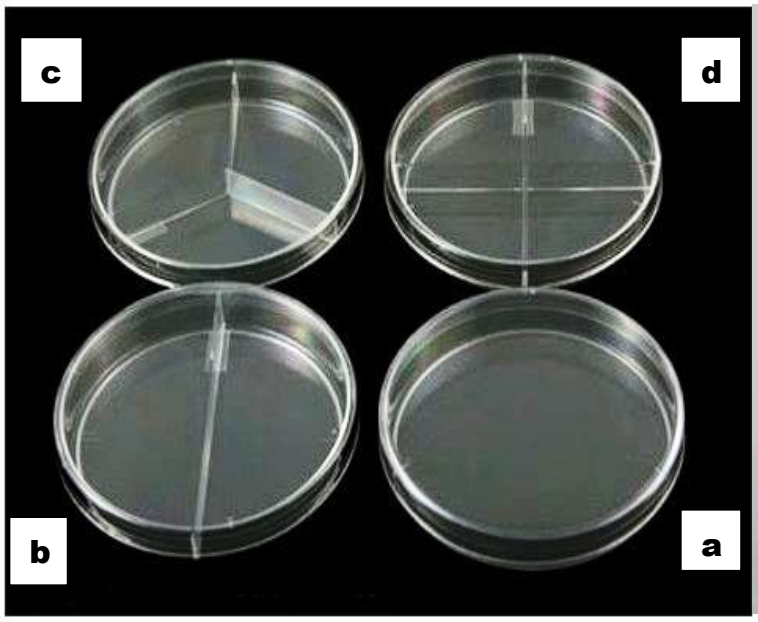
How do you prepare bacterial media?

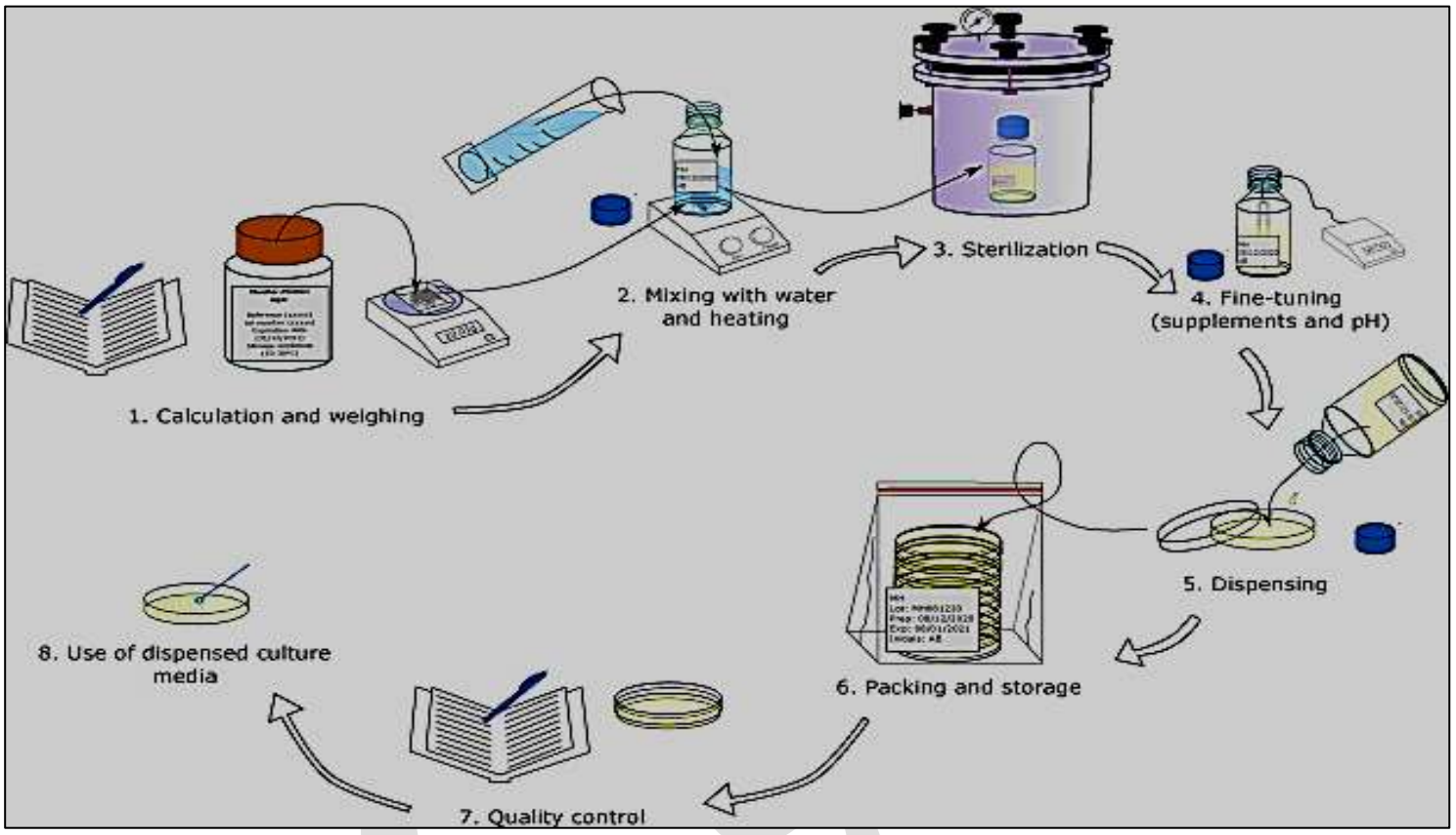
The culture media formulation process involves many steps and must be carried out with care to ***avoid cross contamination as follow:***

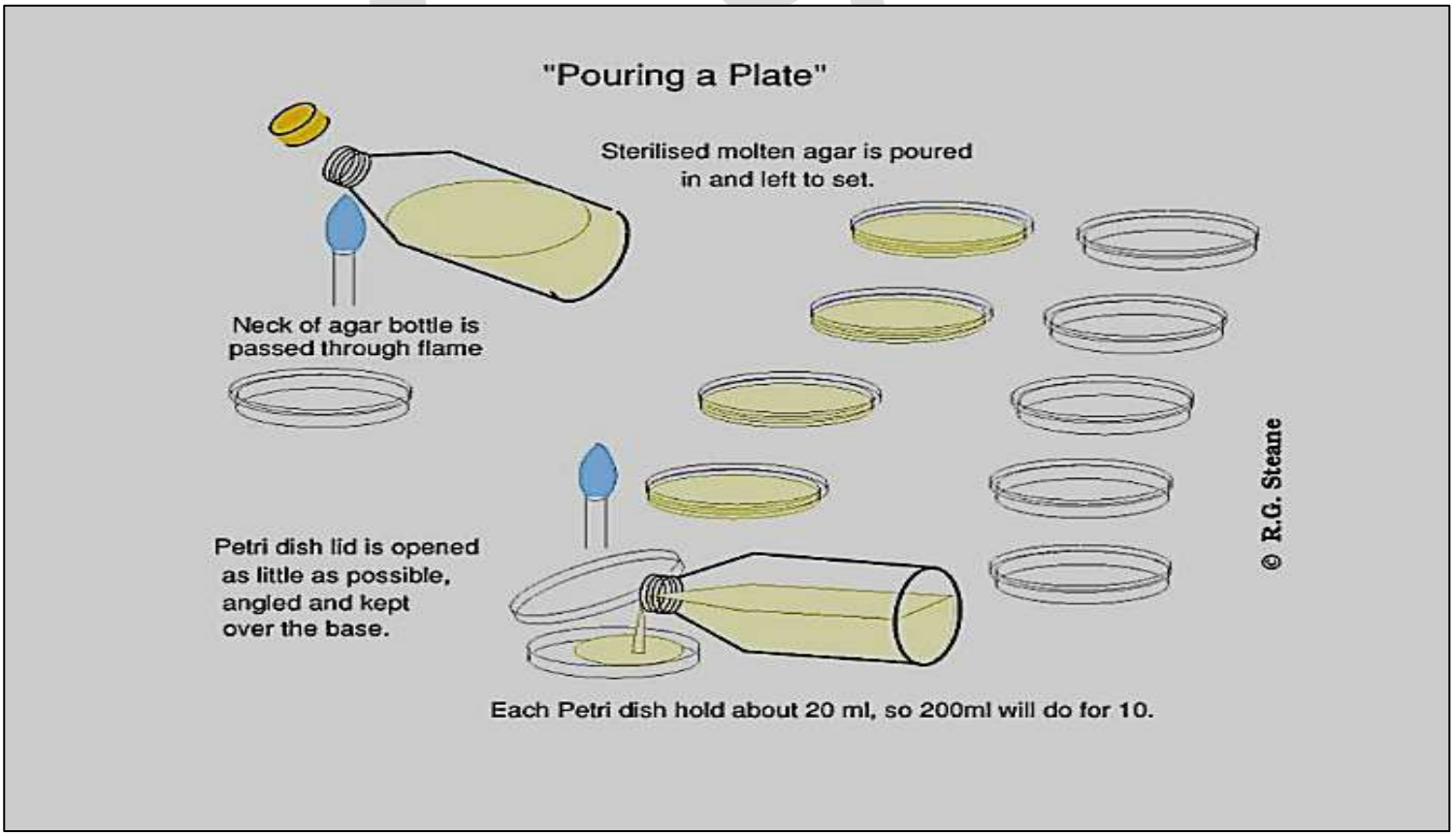
1) Select culture media recipe from database.
2) Recalculate ingredient amounts according to the required culture media volume.
3) Weigh main ingredients into the container.
4) Weigh trace ingredients on a high accuracy balance and add to the container.

5) Pour in deionized water up to around 80% of the required volume.
6) Mix to dissolve the ingredients, gentle heating may be required.
7) Check the pH using a pH meter and modify if necessary.
8) Top up the culture media to the required volume.
9) Label the container.
10) Sterilize in autoclave.
11) Put the media in tubes or on the Petri dishes.
12) Petri dishes are divided according to manufacture into :

a. Complete dishes.
b. Two-part dishes.
c. Triple division dishes.
d. Quadruple plates.




1. Calculation and weighing
2. Mixing with water and heating
3. Sterilization
4. Fine-tuning (supplements and pH)
5. Dispensing
6. Packing and storage
7. Quality control
8. Use of dispensed culture media


"Pouring a Plate"
Sterilised molten agar is poured in and left to set.
Neck of agar bottle is passed through flame
Petri dish lid is opened as little as possible, angled and kept over the base.
Each Petri dish hold about 20 ml, so 200ml will do for 10.
© R.G. Steane

Biochemical Tests and Identification of Bacteria(lab.7)

After the successful cultivation of bacteria in the laboratory, the next step is identification of the genus and species of the cultivated organism.

Methods For The Identification Individual Bacteria

1. Staining Techniques.
2. Biochemical Tests.
3. Serological Tests.
4. Animal Pathogenicity Tests.
5. Nucleic Acid Techniques.

- ❖ Biochemical tests are very useful for the routine identification of pathogenic bacteria for this aim we explain them in this lab.

The validity of the identification of an unknown bacterial culture by its reaction in a range of biochemical tests depends on the *use of a pure culture of the bacteria* for *inoculation of the test media*. Single, well separated colonies grown on a non-selective culture medium should be used as inoculum for the tests.

Common Biochemical Test Used To Differentiate Bacteria:

❖ Sugar Fermentation Test

1. **MacConkey Agar (MacC) Test.**
2. **Mannitol Salt Agar (MSA) Test.**
3. **Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar Test.**
4. **Cystine-Lactose-Electrolyte-Deficient (CLED) Test.**
5. **H_2S Production Test.**

❖ Enzymes Test

1. **Citrate Utilization Test.**
2. **Urease Test.**
3. **Indole Production Test.**
4. **Catalase Test.**
5. **Coagulase Test.**
6. **Oxidase Test.**
7. **Hemolysis Test.**

❖ Sugar Fermentation Test :

1. MacConkey agar

Medium Name ⟶ MacConkey agar

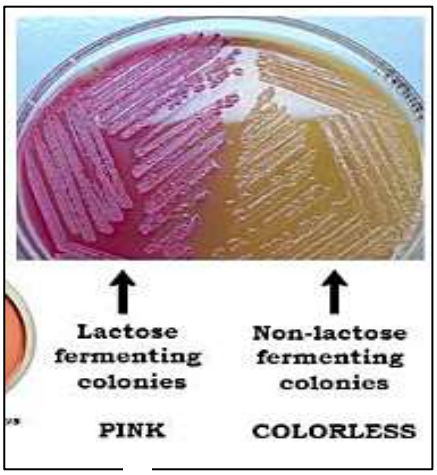
Selective agent ⟶ Crystal Violet + Bile Salts

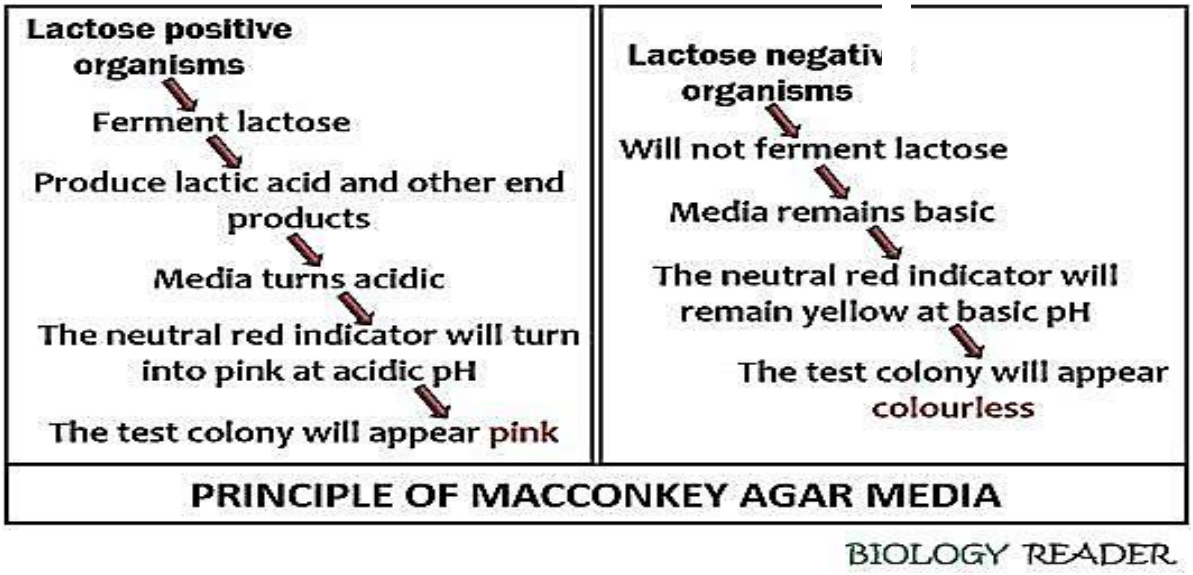
Aim of Use ⟶ used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria.

Substrate Name ⟶ Lactose monohydrate

Indicator Name ⟶ Neutral red+ Crystal Violet

Selective Action ⟶ is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria.




Lactose positive organisms
Ferment lactose
Produce lactic acid and other end products
Media turns acidic
The neutral red indicator will turn into pink at acidic pH
The test colony will appear pink
Lactose negativ organisms
Will not ferment lactose
Media remains basic
The neutral red indicator will remain yellow at basic pH
The test colony will appear colourless
PRINCIPLE OF MACCONKEY AGAR MEDIA
BIOLOGY READER

2. Mannitol Salt Agar (MSA) test

Medium Name ⟶ Mannitol Salt Agar

Selective agent ⟶ Sodium Chloride

Aim of Use ⟶ used as a selective and differential medium for the isolation and identification of *Staphylococcus aureus* from clinical and non-clinical specimens.

Substrate Name ⟶ D-Mannitol

Indicator Name ⟶ Phenol Red

Selective Action ⟶ The 7.5% concentration of sodium chloride results in the partial or complete inhibition of bacterial organisms other than staphylococci.

Mannitol Salt Agar (MSA) for the isolation of *Staphylococcus aureus*

Yellow colonies of *Staphylococcus aureus*

Staphylococcus aureus and *Serratia marcescens* on MSA

3. Thiosulfate citrate bile salts sucrose (TCBS) agar Test

Medium Name ⟶ Thiosulfate citrate bile salts sucrose (TCBS) agar

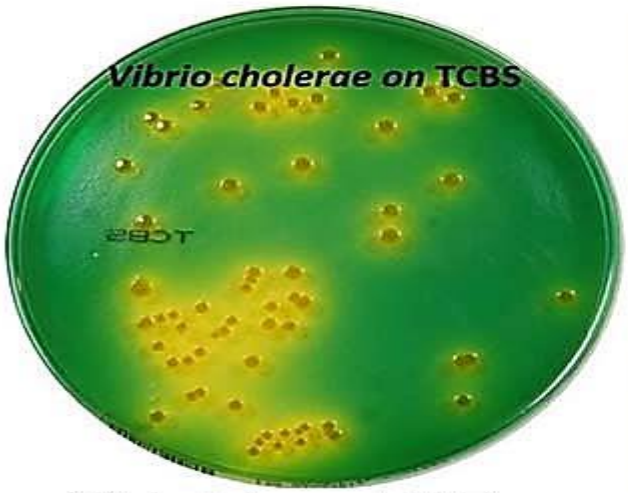
Selective agent ⟶ Sodium thiosulphate, Sodium citrate, Ox Bile

Aim of Use ⟶ a selective as well as differential culture medium used for selective isolation of *Vibrio Spp* from a variety of clinical and nonclinical specimens

Substrate Name ⟶ Sucrose

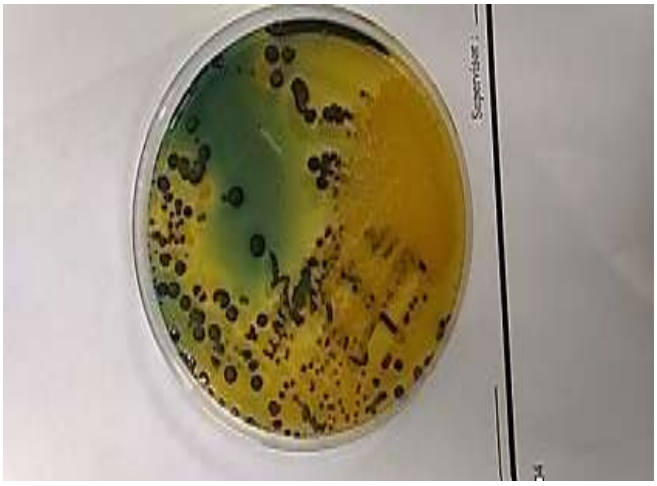
Indicator Name ⟶ Bromothymol blue/ thymol blue

Selective Action ⟶ providing an alkaline pH to inhibit Gram-positive organisms and suppress coliforms.



Vibrio cholerae on TCBS Agar

Vibrio parahaemolyticus on TCBS Agar



4. Cystine-Lactose-Electrolyte-Deficient (CLED) Test

Medium Name ⟶ Cystine-Lactose-Electrolyte-Deficient (CLED)

Selective agent ⟶ L-cystine +Electrolytes

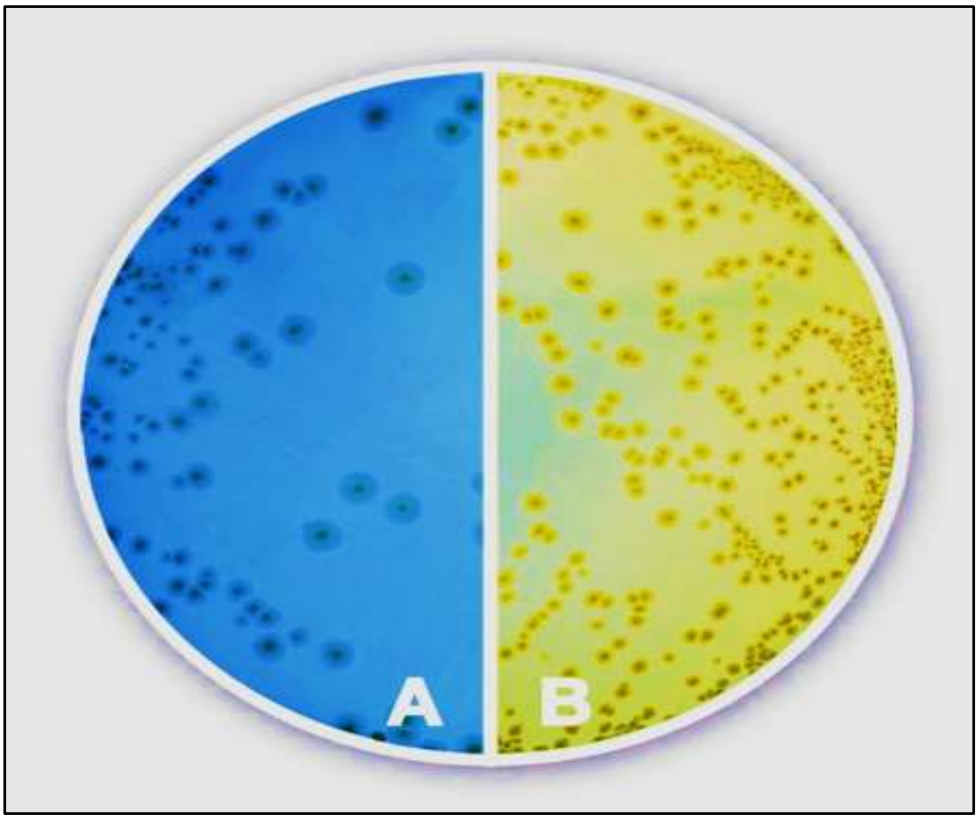
Aim of Use ⟶ diagnostic routine urinary bacteriology as a non-selective medium capable of supporting the growth of most urinary pathogens.

Substrate Name ⟶ Lactose

Indicator Name ⟶ Bromothymol Blue

Selective Action ⟶ L-cystine supports the growth of cysteine- dependent dwarf coliform colony also electrolytes are reduced to restrict the swarming of *Proteus* species

An electrolyte is a medium containing ions that is electrically conducting through the movement of ions. This includes most soluble salts, acids, and bases dissolved in a polar solvent, such as water.



Non-Lactose Fermenters(A) **Lactose Fermenters(B)**

5. H_2S Production Test.

Medium Name ⟶ Kigler Iron Agar(KIA).

Aim of Use ⟶ Differential slope medium between family *Enterobacteriaceae*

Substrate Name ⟶ glucose and lactose

Indicator Name ⟶ Phenol Red, ferric ammonium citrate, and sodium thiosulfate

Selective Action ⟶ fermentation of glucose and lactose and production of hydrogen sulfide

Medium Name ⟶ Xylose –Lysine- Deoxycholate agar (XLDA)

Selective Agent ⟶ Sodium Deoxycholate

Aim of Use ⟶ isolation and differentiation of gram-negative pathogens, particularly salmonella and Shigella.

Substrate Name ⟶ Xylose, lactose, and sucrose

Indicator Name ⟶ Phenol Red, ferric ammonium citrate, and sodium thiosulfate

Selective Action ⟶ fermentation of xylose, lactose, and sucrose, production of hydrogen sulfide

❖ Enzymes test

1. Citrate Utilization Test.

Medium Name ⟶ Simmon's Citrate Agar

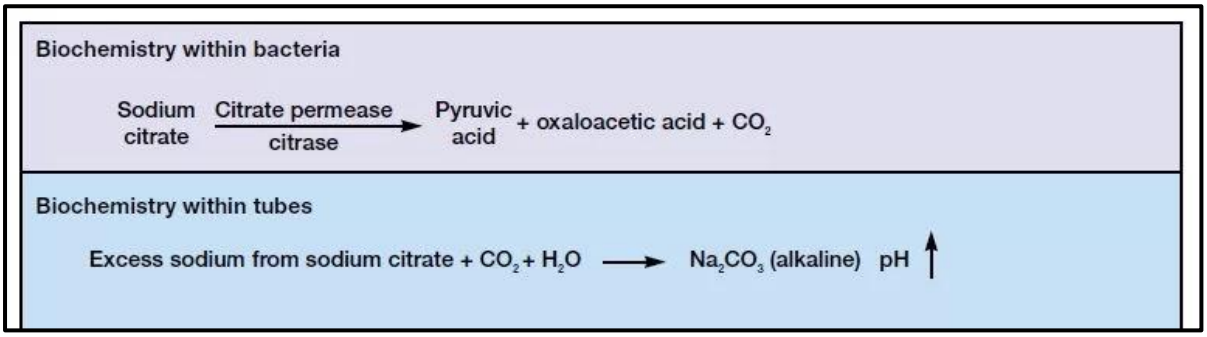
Enzyme Name ⟶ Citrate-Permease

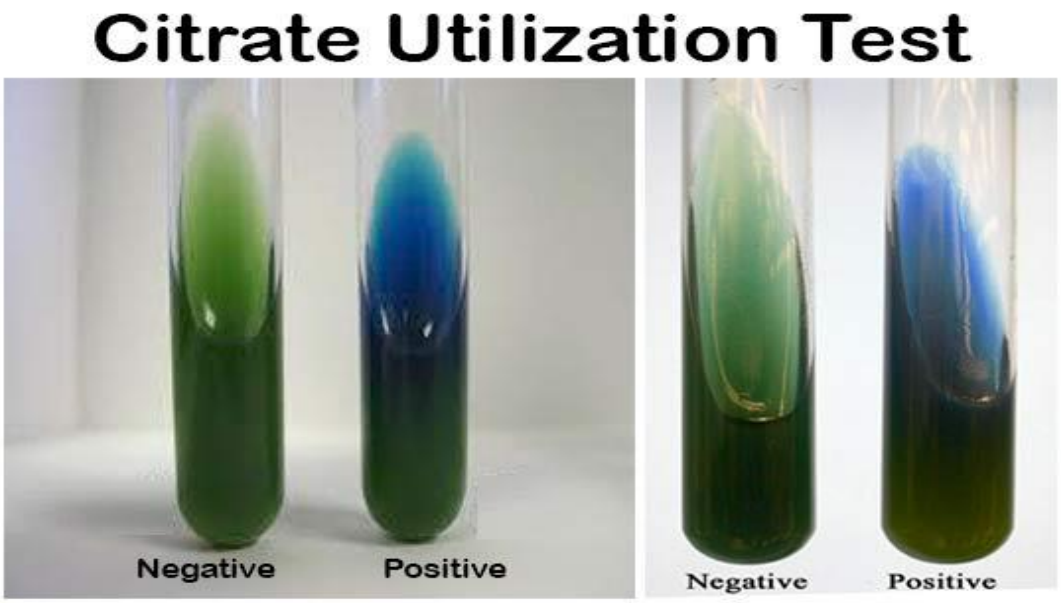
Aim of Use ⟶ test an organism's ability to utilize citrate as a source of energy

Substrate Name ⟶ Sodium Citrate

Indicator Name ⟶ bromthymol blue

Enzyme Action ⟶ Transport the citrate into the cell. And breaks down citrate to oxaloacetate and acetate





2. Urease Test

Medium Name ⟶ **Christensen's Urea Agar**

Enzyme Name ⟶ **Urease**

Aim of Use ⟶ **Detect the ability of bacteria to hydrolyze urea to produce ammonia and carbon dioxide**

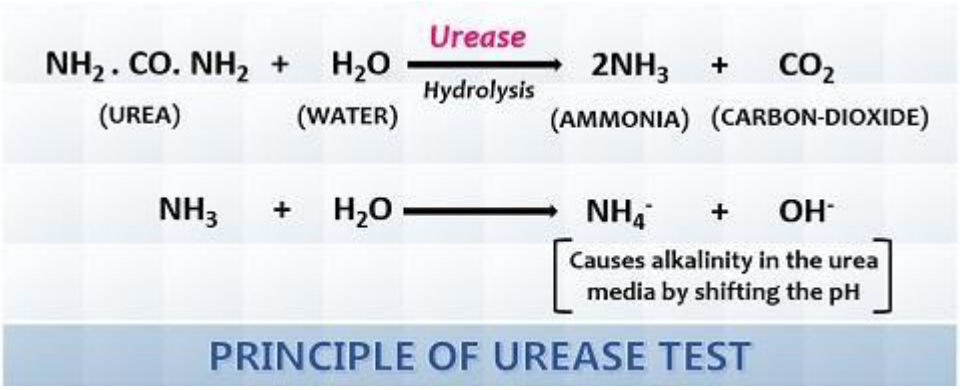
Substrate Name ⟶ **Urea**

Indicator Name ⟶ **Phenol Red**

Enzyme Action ⟶ **catalyzes the hydrolysis of urea, forming ammonia and carbon dioxide**

$(NH2)2CO + 2\ H_2O$ ⟶ $CO2$ + H_2O + $2\ NH_2$

Urea — Urease — Carbon Dioxide — Water — Ammonia



3. Indole Production Test

Medium Name ⟶ Sulfur, Indole, Motility medium (SIM)

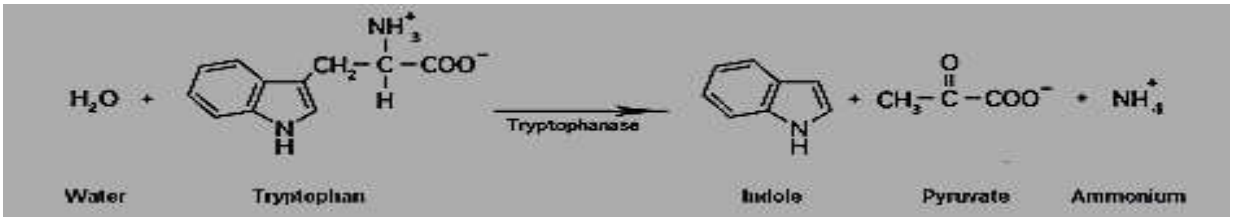
Enzyme Name ⟶ Tryptophanase

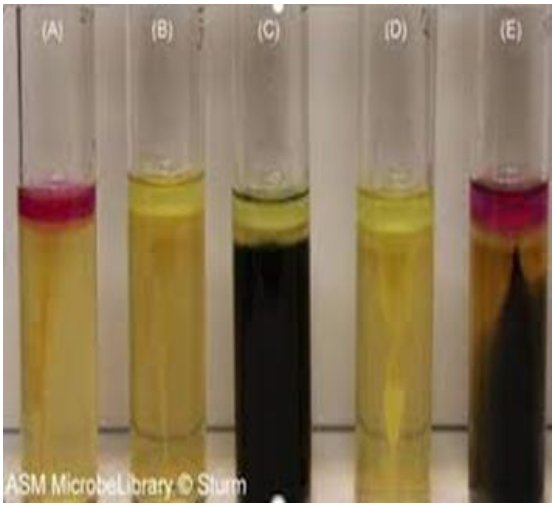
Aim of Use ⟶ Detect the ability of bacteria to convert tryptophan into indole.

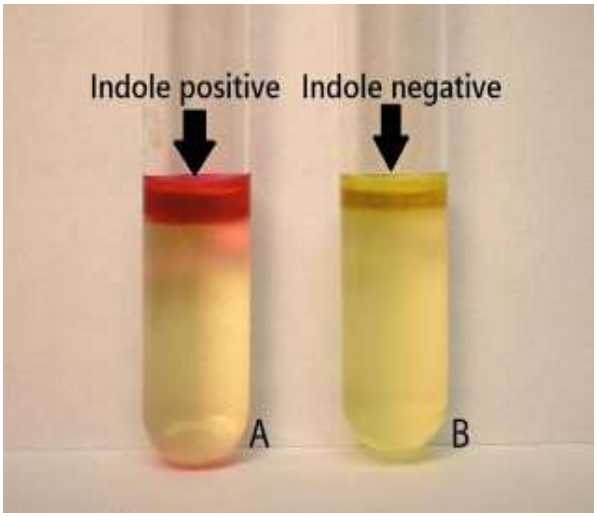
Substrate Name ⟶ Tryptophan

Indicator Name ⟶ indole + Kovac's reagent

Enzyme Action ⟶ break down the amino acid tryptophan with the release of indole.







4. Catalase Test.

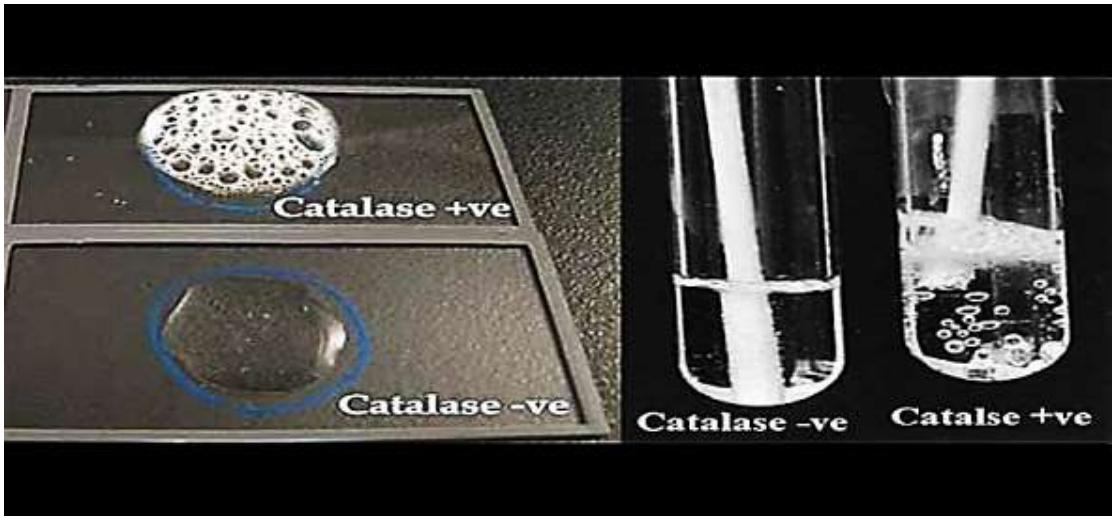
Enzyme Name ⟶ Catalase

Enzyme Importance ⟶ in protecting the cell from oxidative damage by reactive oxygen species

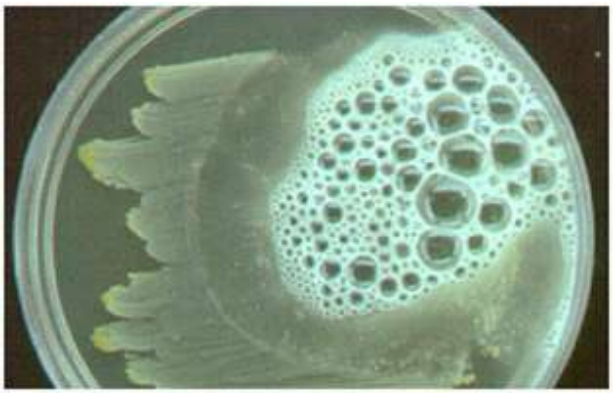
Substrate Name ⟶ Hydrogen Peroxide

Enzyme Action ⟶ break down of toxic H_2O_2 producing oxygen gas and water

- $2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$
- Hydrogen peroxide produce due to the aerobic respiration of the cells and have to be breakdown to prevent it's toxic action on DNA and cell membrane



Slide Method **Tube Method**



Direct Plate Method

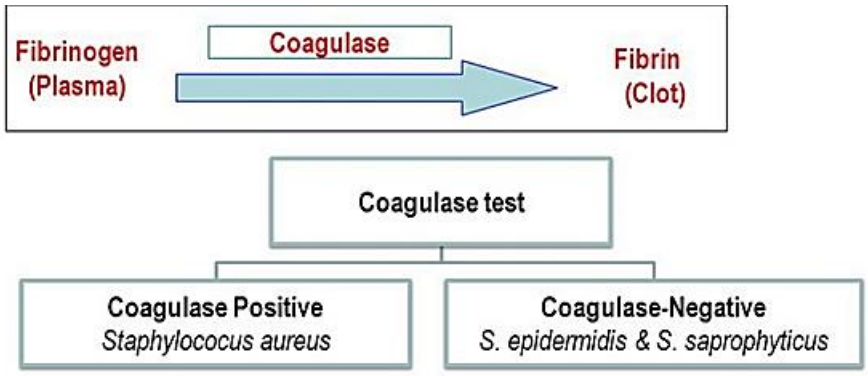
5. Coagulase Test

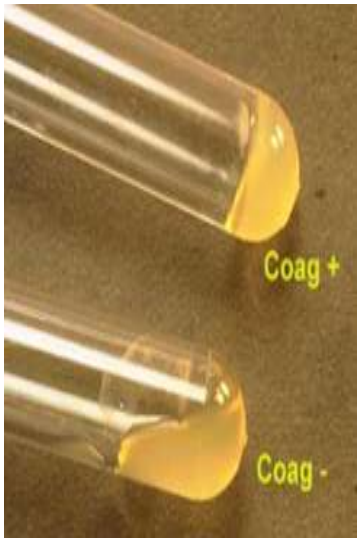
Enzyme Name ⟶ Coagulase

Enzyme Importance ⟶ helps the bacteria evade detection and phagocytosis by the immune system

Substrate Name ⟶ fibrinogen

Enzyme Action ⟶ binds to and activates prothrombin, thereby converting fibrinogen to fibrin and promoting clotting of plasma or blood.





6. Oxidase Test

Enzyme Name ⟶ Cytochrome c Oxidase

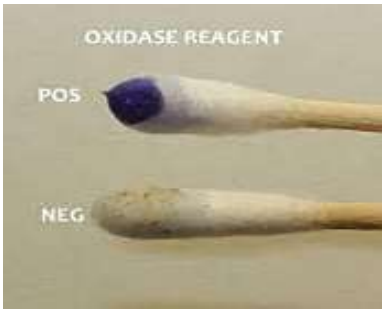
Indicator Name ⟶ Tetramethyl Phenylenediamine Dihydrochloride (TMPD) Reagent

Enzyme Action ⟶ when the reagent is oxidized by Cytochrome c Oxidase, it changes from colorless to dark blue or purple compound.

→ There are present different methods of oxidase test these are includes;

The filter paper test, filter paper spot(oxidase disc or strip) test, direct plate method, test tube method. , and swab method

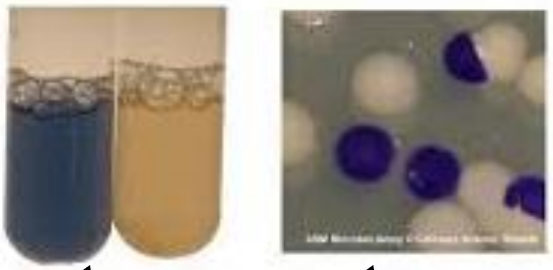
$$2\,\text{reduced cytochrome c} + 2H^{+} + \tfrac{1}{2}O_2 \xrightarrow[\text{oxidase}]{\text{cytochrome}} 2\,\text{oxidized cytochrome c} + H_2O$$



Swab Method

Oxidase Disc Method

Filter Paper Method



Test Tube and Direct Plate Methods

Oxidase positive

Oxidase negative

Impregnated Oxidase Strip Methods

7. Hemolysis tests .

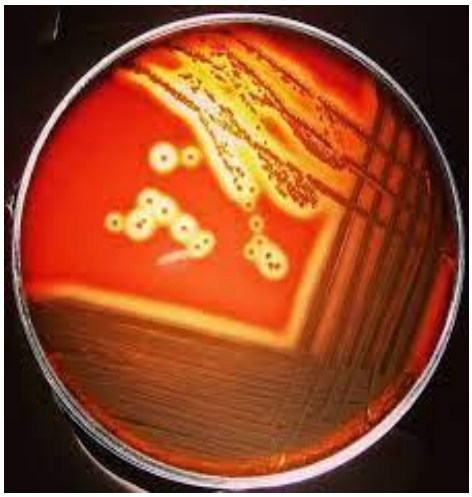
Medium Name ⟶ Blood Agar

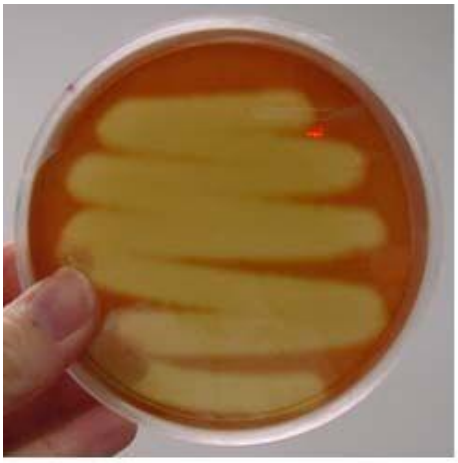
Enzyme Name ⟶ Hemolysin Catalyzes Enzyme

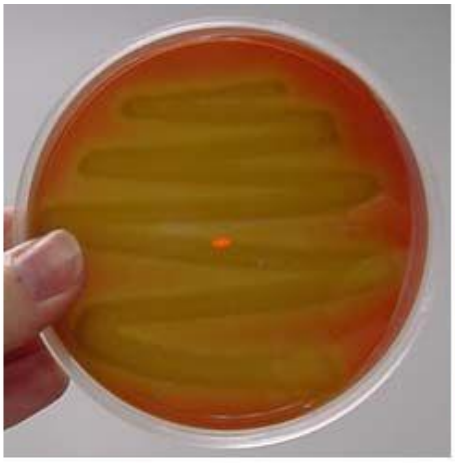
Substrate Name ⟶ Hemoglobin Molecules

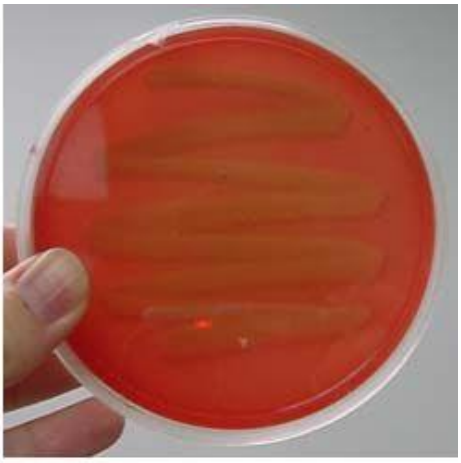
Enzyme Action ⟶ breakdown of blood cells lead to release of hemoglobin from red blood cells to blood plasma. This is a process called hemolysis.

Un-Inoculation blood Medium







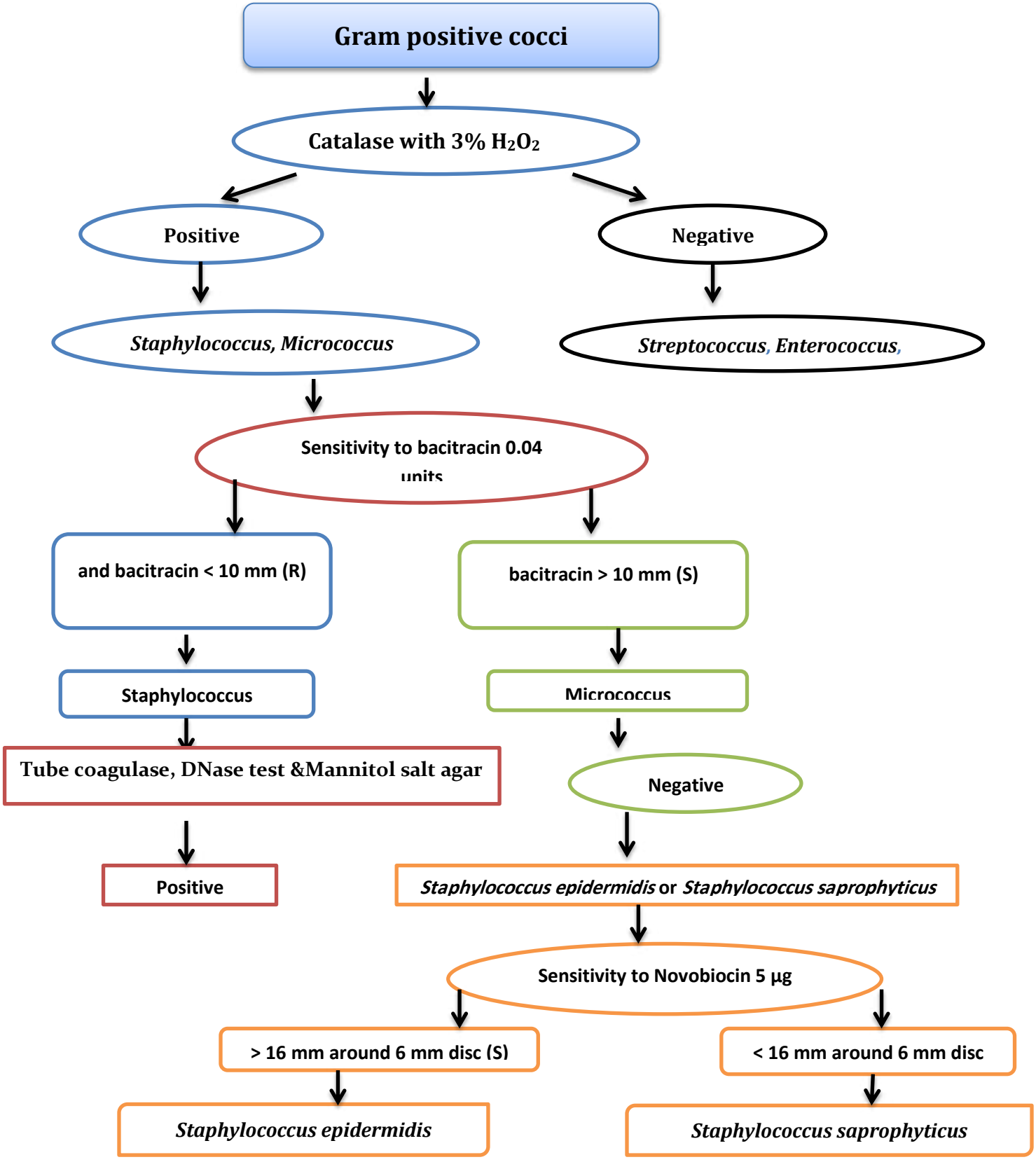


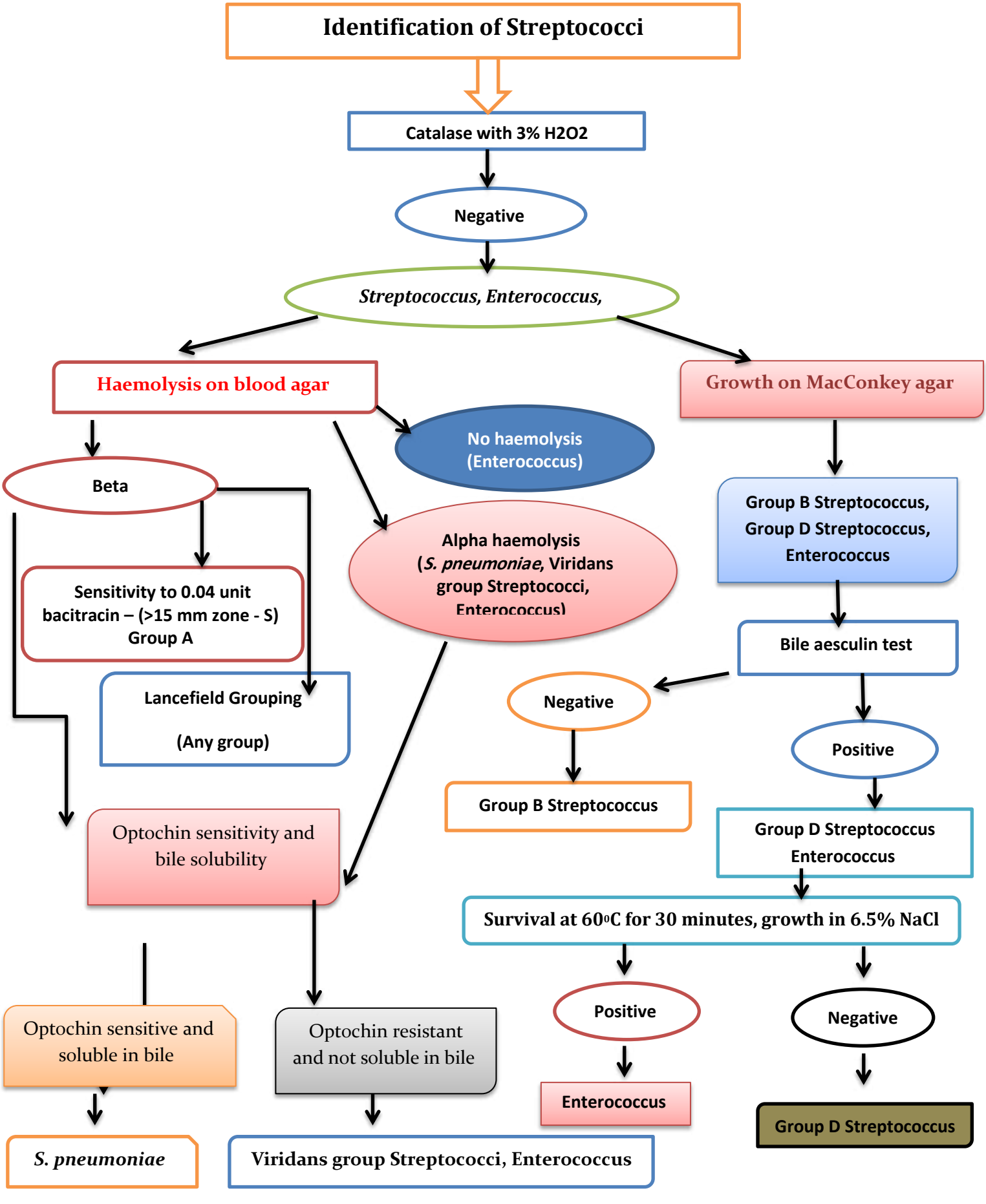
Beta Hemolysis **Alpha Hemolysis** **Gamma Hemolysis**

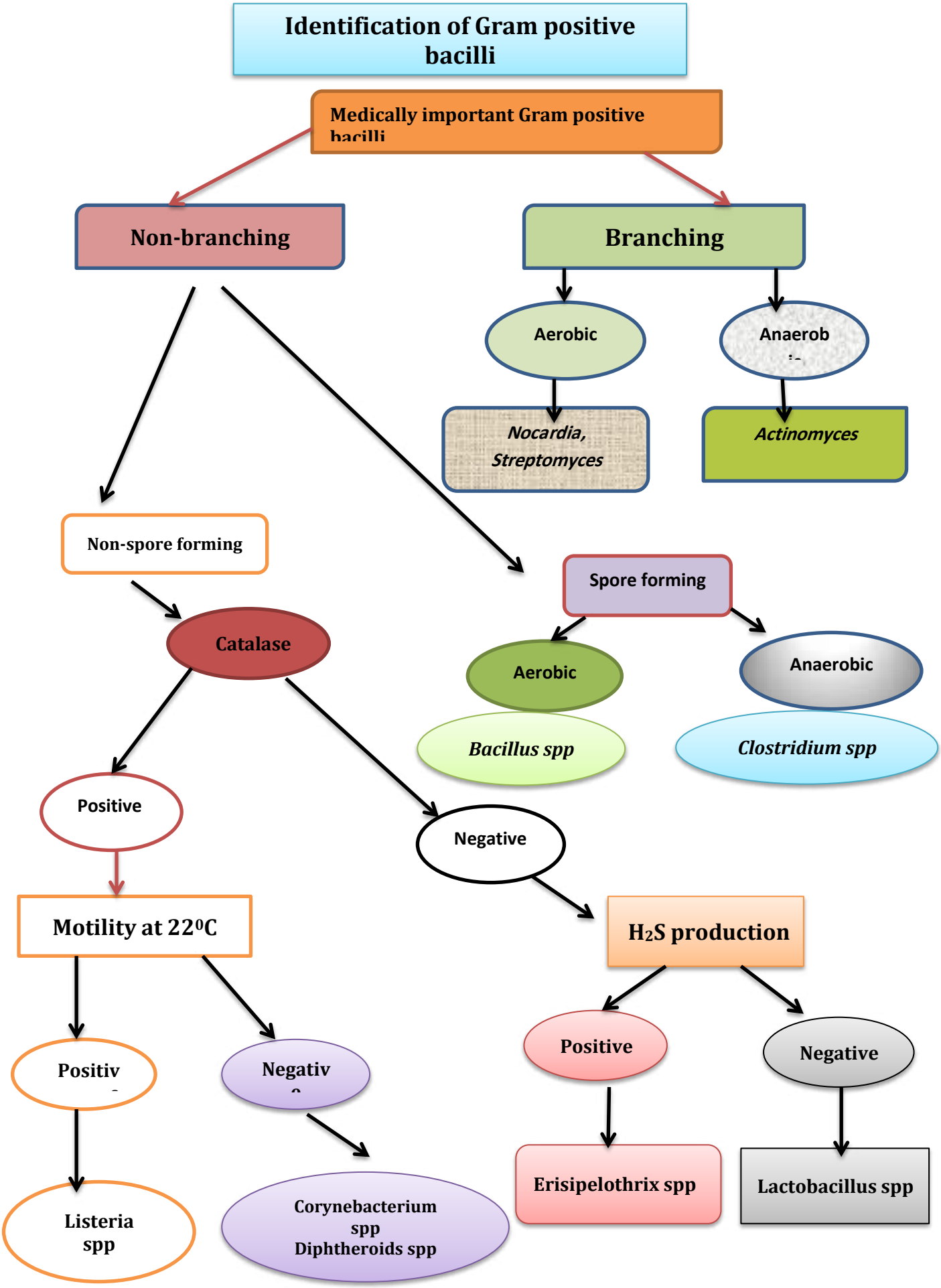
Complete Hemolysis **Partial Hemolysis (Incomplete)** **Non- Hemolysis**

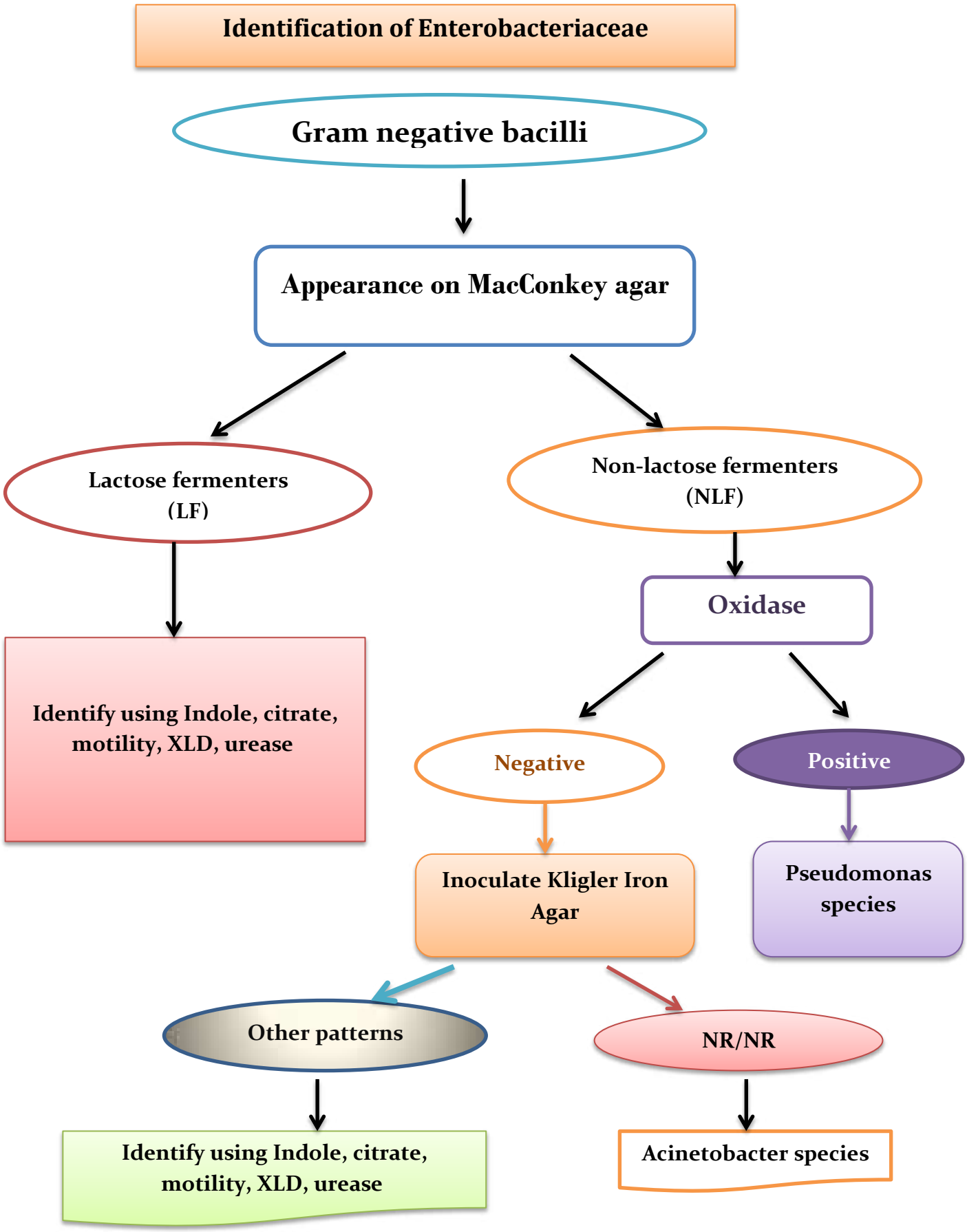
Key to identify Gram Bacteria(lab.8)

Key to identify Gram positive cocci, bacilli & Enterobacteriaceae




Identification of Streptococci
Catalase with 3% H2O2
Negative
Streptococcus, Enterococcus,
Haemolysis on blood agar
Growth on MacConkey agar
No haemolysis (Enterococcus)
Beta
Alpha haemolysis (S. pneumoniae, Viridans group Streptococci, Enterococcus)
Group B Streptococcus, Group D Streptococcus, Enterococcus
Sensitivity to 0.04 unit bacitracin – (>15 mm zone - S) Group A
Bile aesculin test
Lancefield Grouping (Any group)
Negative
Positive
Group B Streptococcus
Group D Streptococcus Enterococcus
Optochin sensitivity and bile solubility
Survival at 60°C for 30 minutes, growth in 6.5% NaCl
Positive
Negative
Optochin sensitive and soluble in bile
Optochin resistant and not soluble in bile
Enterococcus
Group D Streptococcus
S. pneumoniae
Viridans group Streptococci, Enterococcus


Identification of Gram positive bacilli
Medically important Gram positive bacilli
Non-branching
Branching
Aerobic
Anaerob
Nocardia, Streptomyces
Actinomyces
Non-spore forming
Spore forming
Catalase
Aerobic
Anaerobic
Bacillus spp
Clostridium spp
Positive
Negative
Motility at 22⁰C
H₂S production
Positiv
Negativ
Positive
Negative
Listeria spp
Corynebacterium spp Diphtheroids spp
Erisipelothrix spp
Lactobacillus spp


Identification of Enterobacteriaceae
Gram negative bacilli
Appearance on MacConkey agar
Lactose fermenters (LF)
Non-lactose fermenters (NLF)
Identify using Indole, citrate, motility, XLD, urease
Oxidase
Negative
Positive
Inoculate Kligler Iron Agar
Pseudomonas species
Other patterns
NR/NR
Identify using Indole, citrate, motility, XLD, urease
Acinetobacter species

Collection of Clinical Specimens for Microbiological Investigations(lab.9)

INTRODUCTION

Microbiological laboratories aid the clinicians in many ways**.** First and fore most is *the diagnosis of disease process* and *treatment of it*. Hence care should be taken during collection of the specimens at bed sides. In the medical microbiology laboratory, several procedures are carried out for the specimens that reach it, as follows:

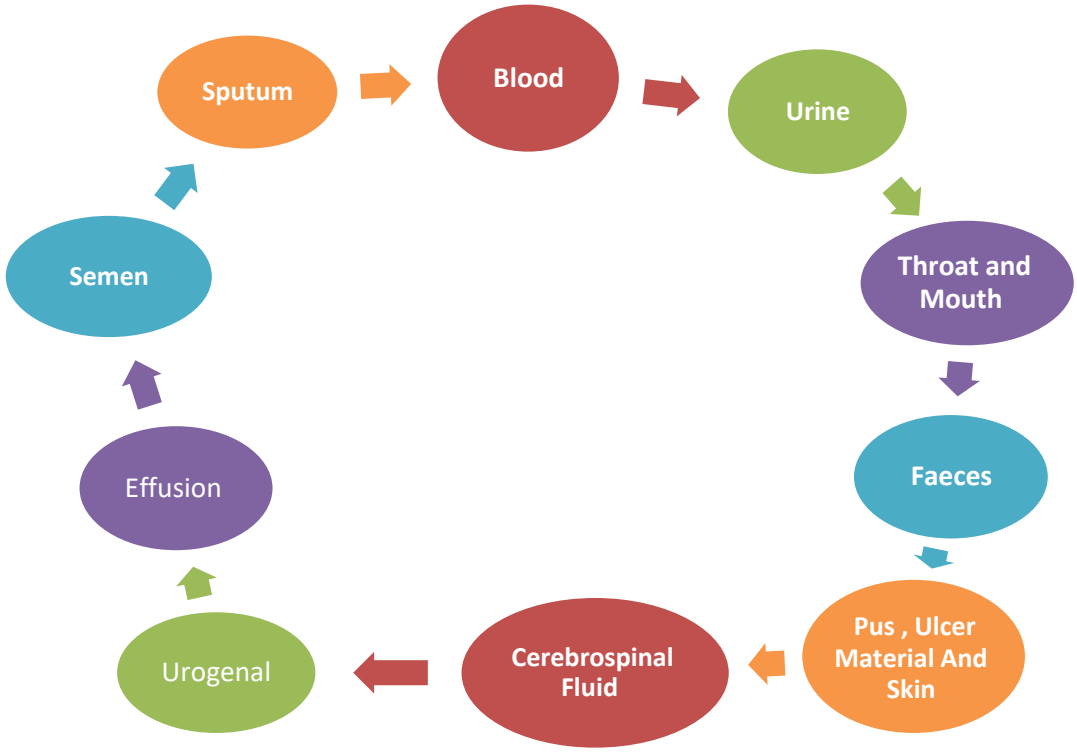
1) Isolation of pathogen that cause the diseases.
2) Diagnosis of infection microorganism.
3) Identification of the best treatment options for infection.
4) The monitoring of antibiotic resistance of antibiotic resistance.
5) Testing for how well a patient is responding to treatment of infection.

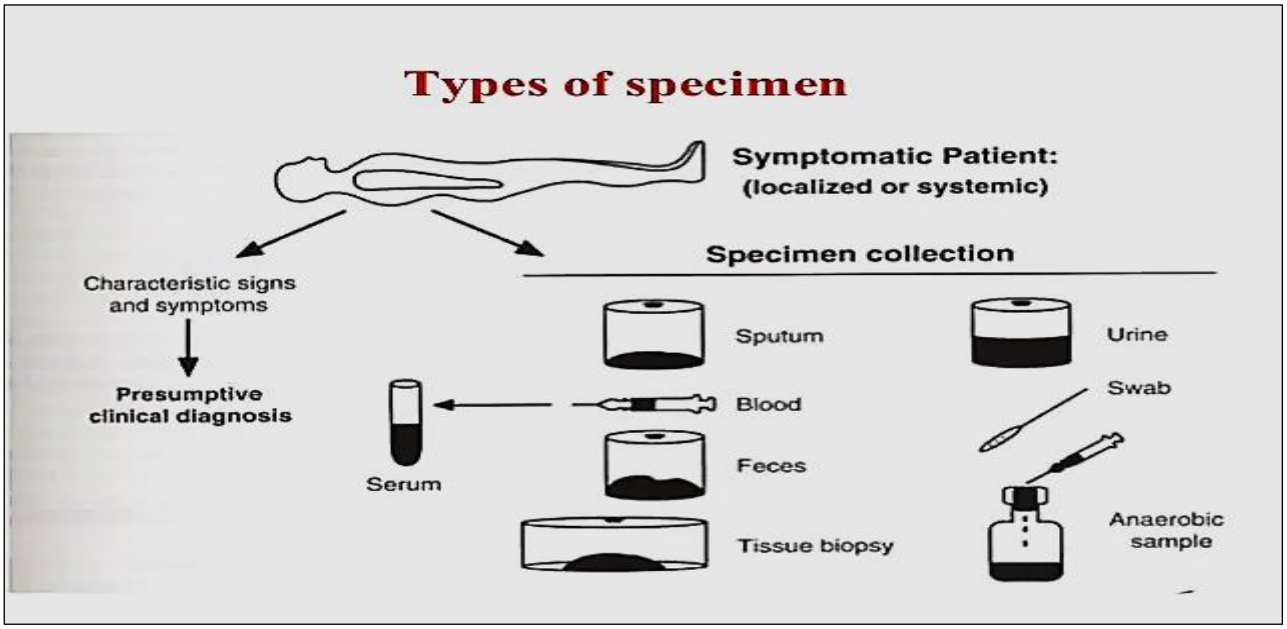
What is specimen for culture?

- Specimen collection is the process of obtaining tissue or fluids for laboratory analysis or near-patient testing. It is often a first step in determining diagnosis and treatment.
- The type of ***specimen*** depends on the location of the suspected infection.

What are the types of specimen collection?

Most commonly these are blood, urine, saliva, sputum, faeces, semen and other bodily fluids, as well as tissue. Some tests can be performed on more than one type of ***specimen***. For example, glucose testing can be performed on blood, urine and other samples





Which specimen should be processed first in microbiology?

If cultures are desired, Microbiology must receive the specimen first to ensure the culturing of a sterile specimen.

- ***Standard precautions and guidelines must be followed, while collecting a clinical sample.***

1. All culture samples (except stool culture) must be collected aseptically in the correct sterile containers provided by the laboratory.
2. The specimens should be from the suspected infective condition.
3. Obtain during acute phase of infection (within 2–3 days for viruses).
4. Sterile swabs and sterile containers should be used to avoid contamination. These tools can be supplied from the Microbiology department.
5. The sample should be in sufficient quantity to decrease the chance of false-negative results.
6. It is always better to send the sample before administration of antibiotic, if not; the antibiotic used should be informed.
7. Collect the specimen with as little contamination as possible to insure that the sample will be representative of the infected site.
8. The specimens should be clearly labelled with patient's name, date of collection, time of collection and hospital number.
9. Collection containers must be closed tightly to prevent leaking of sample during transportation to the laboratory.
10. This sample should be always accompanied by complete laboratory requisition forms with complete details, like patient's name, age, sex, nature of specimen, clinical features and its duration with probable clinical diagnosis.

What are the steps in specimen collection?

There are four steps involved in obtaining a good quality specimen for testing:

a. Preparation of the patient.
b. Collection of the specimen.
c. Processing the specimen.
d. Storing and/or transporting the specimen.

1. Urine collection specimen

1. Urine specimens should be transported to the laboratory immediately with-in one hour. If that is not possible they should be kept in the refrigerator for not more than 24 hours' time.
2. **Collect 2-10ml midstream urine in a sterile container**.
3. Early morning urine is preferable to enable the organism to multiply in the bladder before collection. If not possible a period of 3 hours must elapse after the last urination.
4. Discard the first portion of voided urine and collect the midstream urine directly into a sterile bottle.
5. <u>Catheter tip:</u> Cleanse skin around catheter site with alcohol. Aseptically remove and clip 5cm distal tip catheter into **sterile container** and send immediately the lab.

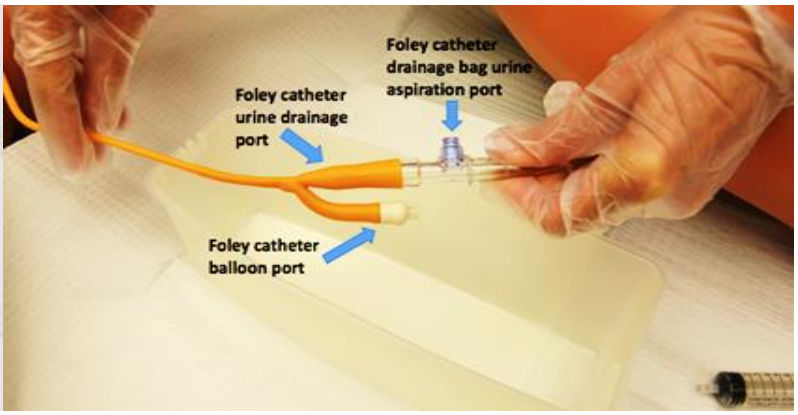
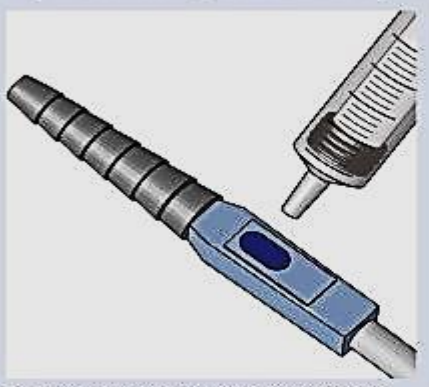
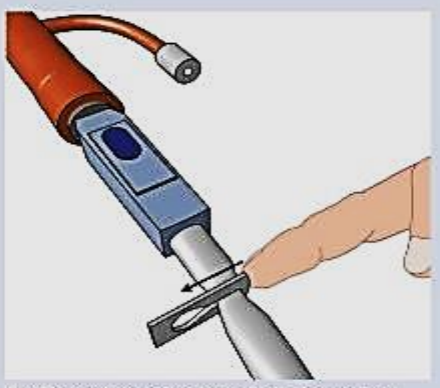


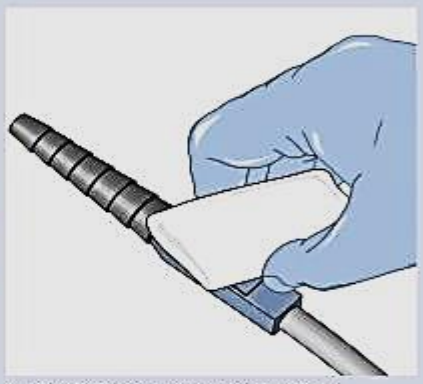
Fig 1. **Collecting a catheter specimen of urine**



1a. A sampling port can be found on the tubing of the catheter drainage bag – urine should only be obtained from this point.

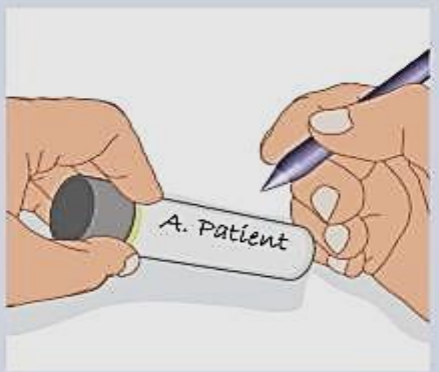


1b. Clamp the catheter below the port so that urine can collect above it in the tubing. Some catheter bags have an integral clamp.



1c. Swab the sampling port with an alcohol-impregnated swab following local policy to reduce the risk of cross infection and contamination of the specimen.

1d. Insert the syringe tip into the sampling port and withdraw the urine following manufacturer's instructions.

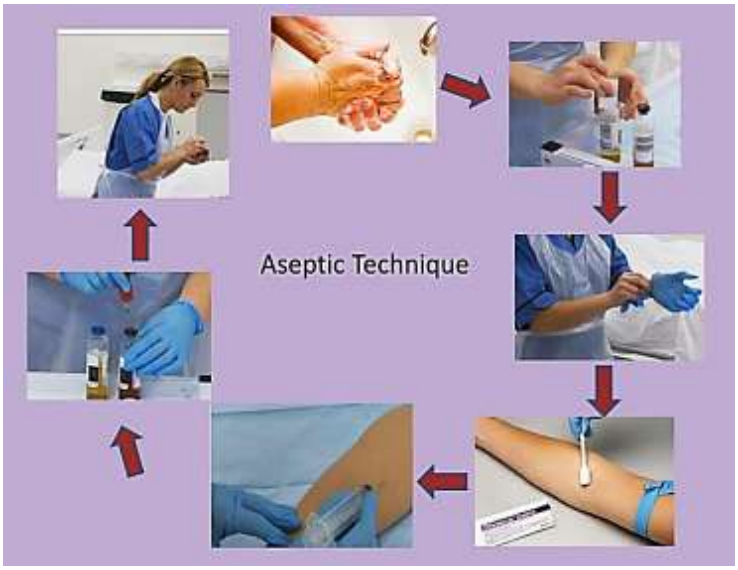


1e. Place sample in the specimen pot, avoiding contact with the syringe. Secure top to prevent leakage and contamination, then label, place in a specimen bag and seal.

1f. If the sample is taken from a catheter valve, the valve must be cleaned with an alcohol-impregnated swab first to reduce the risk of cross infection.

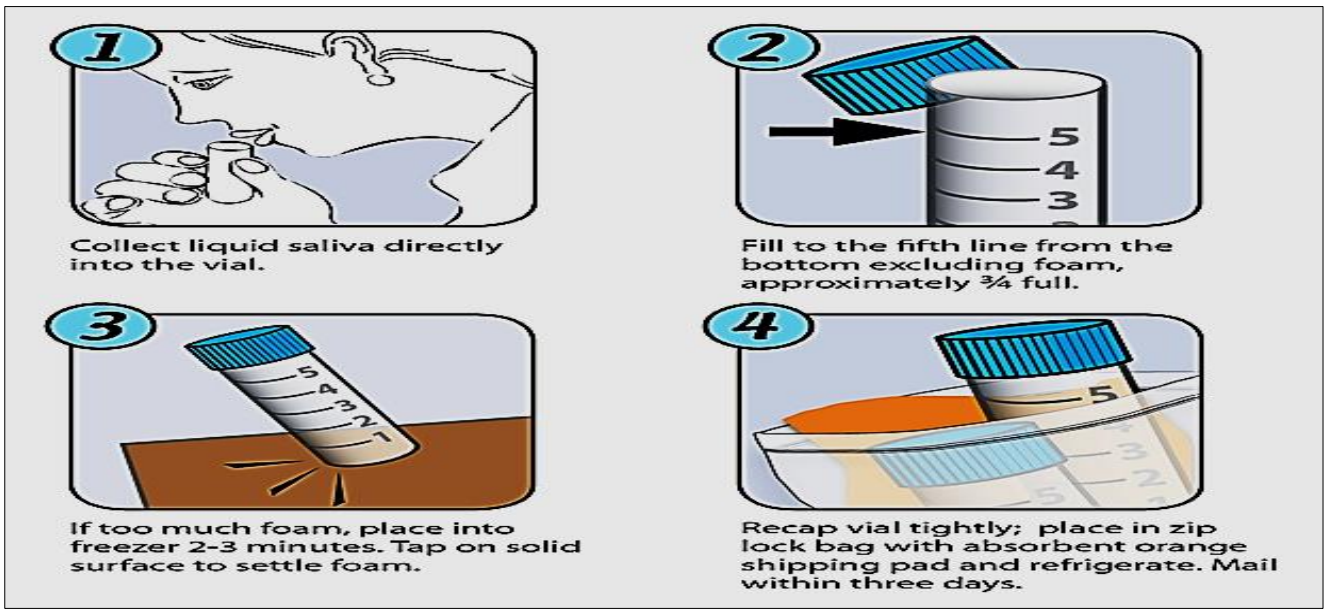
2. Blood collection specimen

The following apparatus are required, Sterile 10 ml syringe, 21 gauge needle, antiseptic solution to clean the skin, spirit lamp and Media for culture of different organisms.



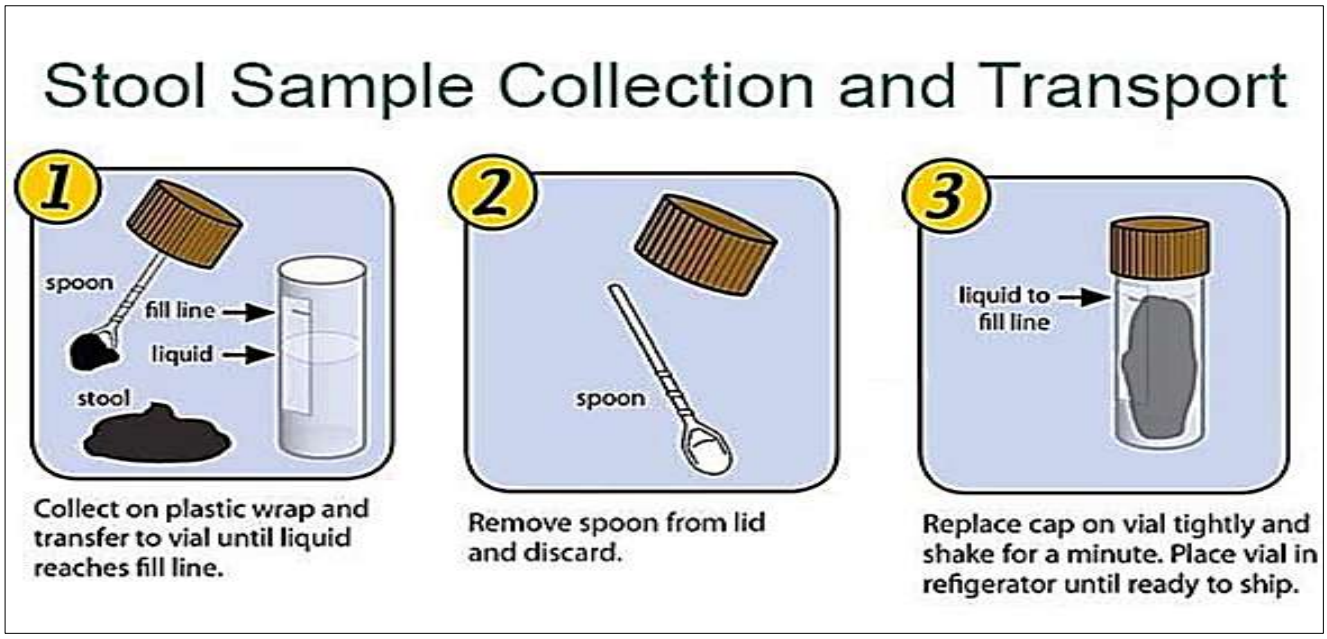
3. Sputum and Saliva Collection Specimen

Sputum is preferably collected when the patient first wakes up in the morning. Gargle mouth before sputum collection.


1
Collect liquid saliva directly into the vial.
2
5
4
3
Fill to the fifth line from the bottom excluding foam, approximately ¾ full.
3
If too much foam, place into freezer 2-3 minutes. Tap on solid surface to settle foam.
4
5
Recap vial tightly; place in zip lock bag with absorbent orange shipping pad and refrigerate. Mail within three days.

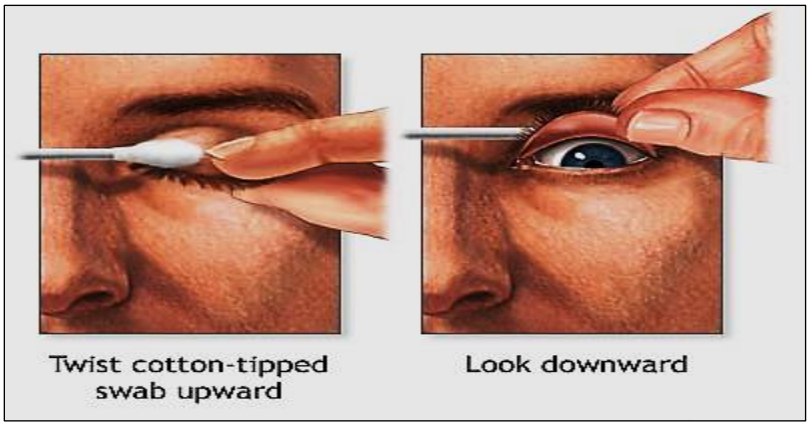
4. Stool collection specimen

It should be collected in sterile containers and immediately sent to the laboratory.


Stool Sample Collection and Transport
1
spoon
fill line
liquid
stool
Collect on plastic wrap and transfer to vial until liquid reaches fill line.
2
spoon
Remove spoon from lid and discard.
3
liquid to fill line
Replace cap on vial tightly and shake for a minute. Place vial in refigerator until ready to ship.

5. Eye collection specimen

Collect the exudate with separate sterile swabs.

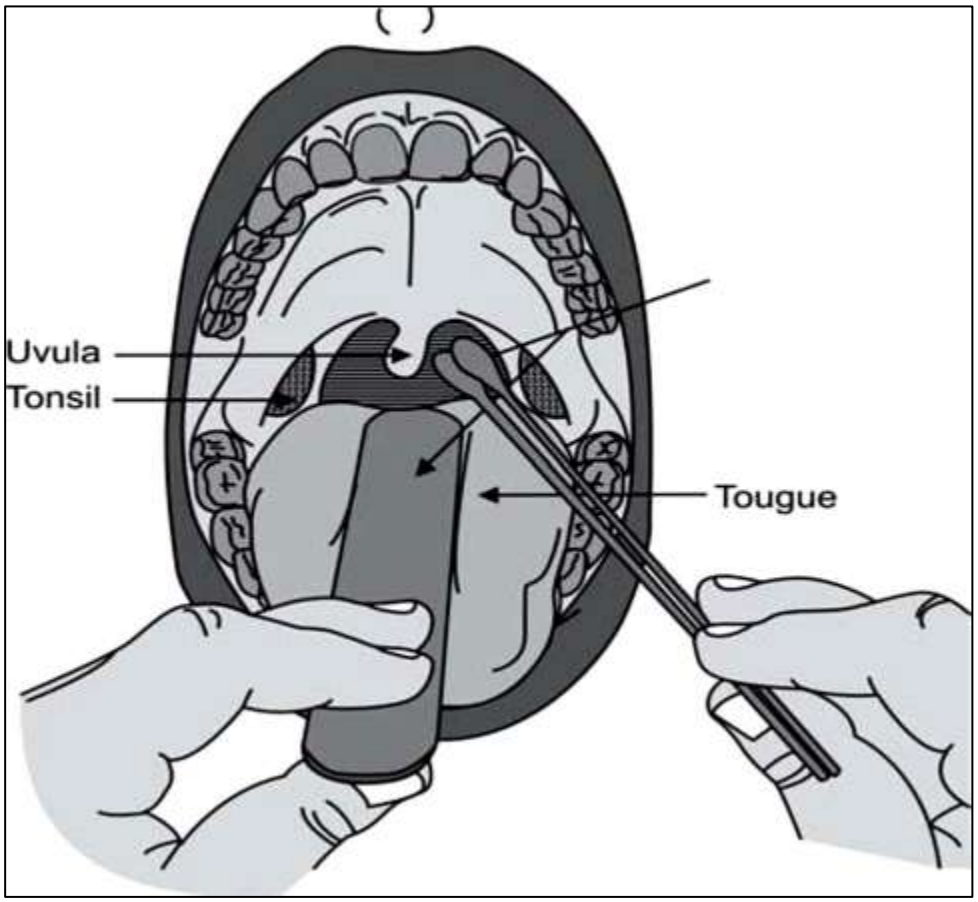


Transport medium

6. Throat collection specimen

Make it a point always to send two swabs to the laboratory—One for smear and one for culture.

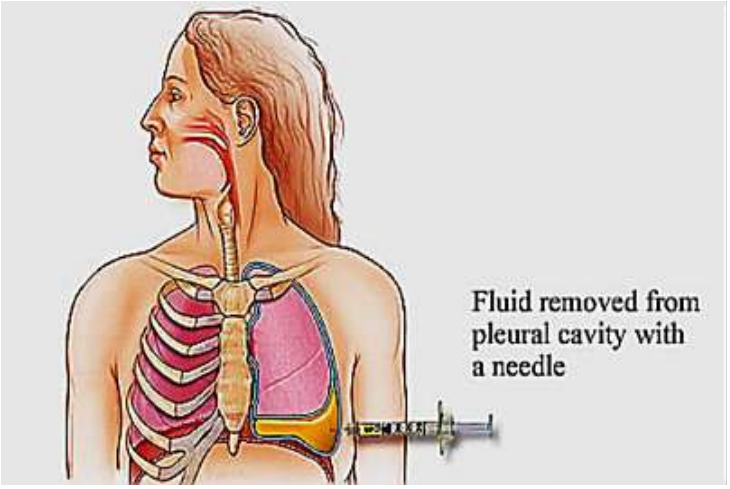
Throat Specimen Collection Instructions
1. Insert swab in posterior pharynx and tonsillar areas.
2. Rub swab over posterior pharynx and bilateral tonsillar pillars; avoid tongue, teeth, and gums.
3. Immediately place in sterile tube containing transport medium.

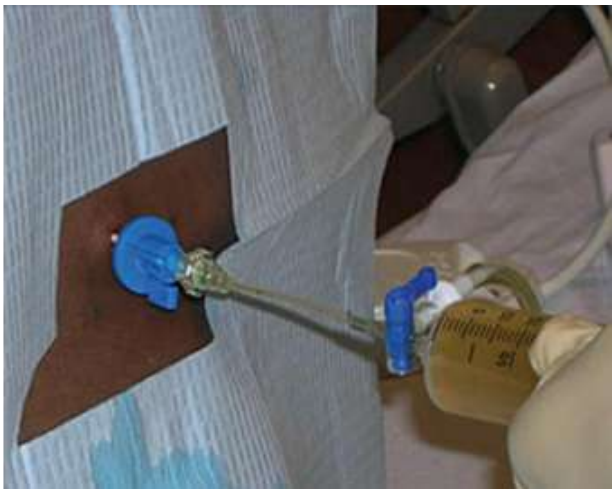


Throat swab collection

7. Effusion Specimen Collection

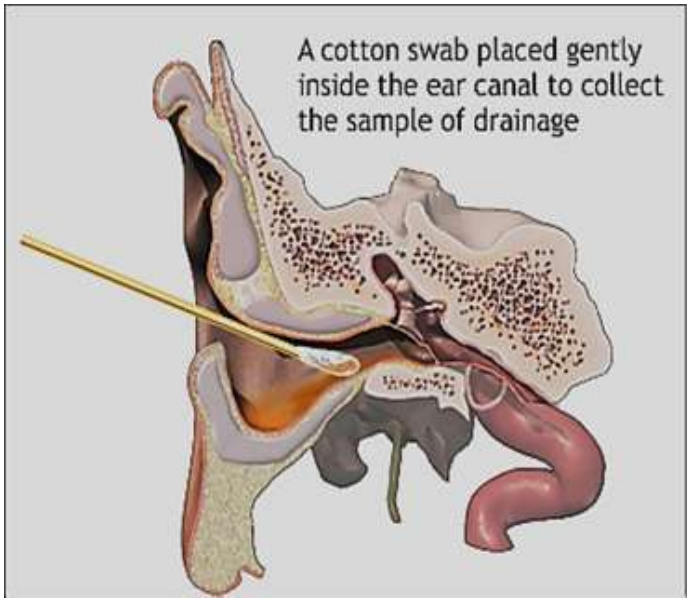
Pus from close abscess can be collected by sterile syringe and needle, by aspiration, afterwards sterile swab is used. Biopsy and autopsy specimens should be sent to the laboratory in sterile containers.





8. Ear specimen collection

1. Two swabs should be taken, one for the mycological examination another for the bacterial cultures.
2. The external meatus is cleaned with a dry swab moistened with sterile saline. Let the site dry before sampling.
3. Pass a swab gently into the external canal and collect whatever exudate that is found there.



Examples of Some Types of Samples and the Culture Media Used for Them

- **In this step, the medical technologist should be:**
  - ✓ Culture the sample on media, on which a bacteria are grow
  - ✓ For each sample, there are a specific type of culture media

No.	Sample	Type of culture media
1-	Eye swab	Blood agar + Chocolate agar + MacConkey agar
2-	Nasal swab	Blood agar + Chocolate agar + MacConkey agar
3-	Urethral swab	Blood agar + Chocolate agar + MacConkey agar
4-	Seminal fluid	Blood agar + Chocolate agar + MacConkey agar
5-	Urine	Blood agar + CLED
6-	Throat swab	Blood agar
7-	Rectal swab	Alkaline Peptone Water + TCBS

The most important routine culture media used to isolate pathogenic bacteria:

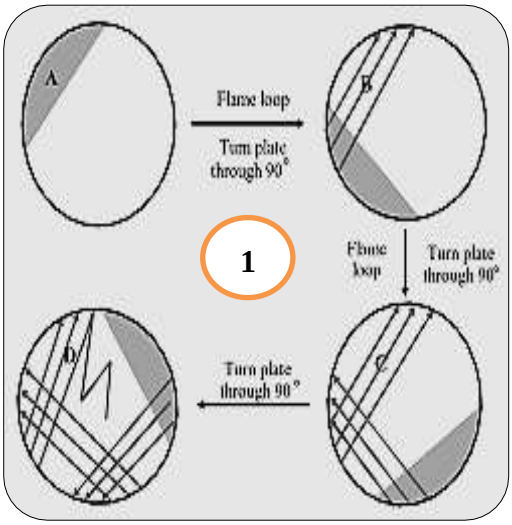
1. *Transport Medium(for Deliver any sample)*→ Stuart's Transport Medium and Amie's transport medium
2. *Blood Agar.*
3. *Chocolate Agar.*
4. *MacConkey's Agar(MaCC) .*
5. *Robertson Cooked Meat (RCM) medium*→ *for anaerobic bacteria*

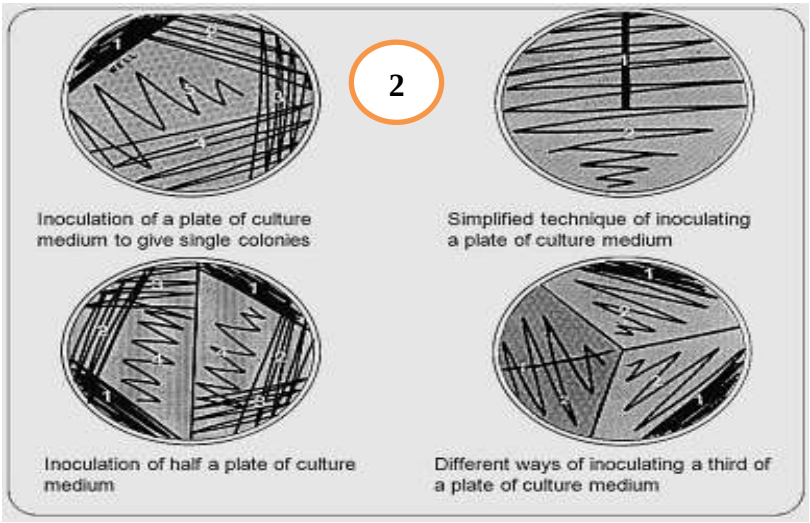
Method of inoculating the solid culture media

For obtaining the isolated colonies streaking method is used, the most common method of inoculating an agar plate is streaking *by loop or swab*

Types of inoculating as you see in picture (2)

Inoculating complete plate for giving single colonies, simplified technique for urine sample, plate half inoculating, and plate third inoculating.




2
Inoculation of a plate of culture medium to give single colonies
Simplified technique of inoculating a plate of culture medium
Inoculation of half a plate of culture medium
Different ways of inoculating a third of a plate of culture medium